# AxiChrom™ 300-1000 columns User Manual





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# 1 Introduction

# 1.1 General introduction

AxiChrom process column family has been designed to deliver reproducible results from process development to production scales. This is facilitated by the innovative Intelligent Packing where UNICORN™ software, ÄKTA™ systems and AxiChrom columns work together to facilitate a convenient operation for packing of the bed via axial compression.

The AxiChrom family of process column comprises the following columns:

- AxiChrom 300
- AxiChrom 400
- AxiChrom 450
- AxiChrom 600
- AxiChrom 800
- AxiChrom 1000

The collective name AxiChrom 300-1000 refers to all columns in the family.

Columns are available in two tube lengths, allowing maximum bed heights of 300 and 500 mm, respectively. The columns are validatable and the construction materials comply with the ASME BPE standards. They are fully traceable back to the source for biopharmaceutical production. All wetted polymer and elastomeric parts are classified according to USP Class VI, 21 CFR Part 177 and are animal origin free, or comply with the conditions in the CPMP Note for Guidance (EMEA/410/01).

The images below show a 600 column (left) and an AxiChrom Master unit (right).





# 1.2 Scope of this User Manual

This User Manual is designed as a general introduction and guide to using AxiChrom 300-1000 columns. It is recommended that the user reads the complete Operating Instructions before using the column. Information concerning safety precautions is presented in this manual in conjunction with general information about the features and operation of the column.

#### Specifically, the instructions cover:

Chapter 1 General introduction, intended use of the columns, safety in-

structions, and labeling.

Chapter 2 AxiChrom 300-1000 columns specifications, including principles

of column function and column specifications.

Chapter 3 Preparation of a column for use, including unpacking, installa-

tion, column and system setup, use of AxiChrom Master and

instructions for cleaning the column before use.

Chapter 4	Preparations required before packing the column, including slurry preparation and priming the column.
Chapter 5	Overview of the Intelligent Packing procedure and instructions on how to pack the column using UNICORN or AxiChrom Master interface.
Chapter 6	Guidance how to evaluate the performance of the column and perform HETP calculations. $ \label{eq:collection} % \begin{center} center$
Chapter 7	Instructions for performing CIP, Sanitization-in-place (SIP), unpacking the column using UNICORN or Master interface and shutting down the column and Master.
Appendices	Spare parts information and exploded diagrams. Tubing kits and connections. Packing buffers and recommended packing factors. Column and crate weights and dimensions. Decontamination report.

# **Associated documentation**

The following documentation is provided with the AxiChrom 300-1000 columns.

Manual name	Document num- ber	Description
AxiChrom 300-1000 columns User Manual	28-9562-89	This manual
AxiChrom 300-1000 columns Operating Instruc- tions	28-9562-90	Safety information and instruc- tions for installation, operation and maintenance. Includes a CD with translations into local languages
AxiChrom columns Site Preparation Guide	Configured for individual columns	Guidelines and requirements regarding the installation site and how to place the column
AxiChrom 300-1000 columns Read Me First	28-9562-91	Instructions on how to unpack the column from the delivery package
Product documentation in binder and on a CD	Configured for individual columns	A CD containing spare parts lists, diagrams, material certificates, etc.

#### Additional documentation on request

- Additional printed versions of documentation available electronically: spare part lists, diagrams and material certificates, etc.
- Packing recommendations, applications and Sanitization-in-place studies, and other supporting documentation

#### Other reference documents

Other documents that should be referred to before using the column:

- ÄKTAprocess User Manual
- ÄKTAprocess Operating Instructions
- UNICORN User Reference Manual for ÄKTAprocess



#### NOTICE

Ensure that the product file is kept updated and together with the product documentation. The file should contain information about inspections, performed maintenance, exchanged spare parts and any other important information for safe operation.

# 1.3 Important user information

# Read this before using AxiChrom columns



All users must read the entire AxiChrom 300-1000 columns *Operating Instructions* before installing, using, or maintaining the instrument. Always keep the Operating Instructions at hand when using AxiChrom 300-1000 columns.

Do not operate AxiChrom 300-1000 columns in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury, and you may cause damage to the equipment.

#### Intended use

The AxiChrom family of process columns has been designed for low pressure chromatographic separation of biomolecules such as proteins, peptides and oligonucleotides in GMP-regulated environments. AxiChrom columns are intended for production use only and should not be used for diagnostic purposes in any clinical or *in vitro* procedures.

The columns are not suitable for operation in a potentially explosive atmosphere or for handling flammable liquids. If the columns are used for purposes other than those specified in the user documentation, safe operation and the protection provided by the system may be impaired.

# Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

#### **Warnings**



#### WARNING

**WARNING** indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

#### Cautions



#### CAUTION

**CAUTION** indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

#### **Notices**



#### NOTICE

**NOTICE** indicates instructions that must be followed to avoid damage to the product or other equipment.

#### Notes and tips

**Note:** A Note is used to indicate information that is important for trouble-free and

optimal use of the product.

**Tip:** A tip contains useful information that can improve or optimize your procedures.

# **Typographical conventions**

Software items are identified in the text by **bold italic** text. A colon separates menu levels, thus **File:Open** refers to the **Open** command in the **File** menu. Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

# 1.4 Regulatory information

This section describes the directives and standards that are fulfilled by the AxiChrom 300-1000 columns and AxiChrom Master.

## **Manufacturing information**

Name of manufacturer	Address of manufacturer	
GE Healthcare Bio-Sciences AB	GE Healthcare Bio-Sciences AB	
	Björkgatan 30, SE-751 84 Uppsala, Sweden	

Other regulatory information may be found in the Declaration of Conformity supplied with the product.

# **CE Conformity**

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards. A copy of the Declaration of Conformity is supplied with the product.

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive
97/23/EC	Pressure Equipment Directive (PED)

# International standards

This product is designed in accordance with the requirements of the following standards and regulations:

Standard	Description	Notes
EN 61010-1, IEC 61010-1, UL 61010-1, CAN/CSA C22.2 No 61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use	EN standard is harmonized with EU directive 2006/95/EC
EN 61326-1 (Emission according to CISPR 11, Group 1, class A)	EMC emissions and immunity requirements for electrical equipment for measurement, control and laboratory use	EN standard is harmonized with EU directive 2004/108/EC
EN ISO 12100	Safety of machinery, General principles for desgn, Risk assessment and risk reduction.	EN ISO standard is harmonized with EU directive 2006/42/EC
ASME BPVC VIII, div 1	Boiler and Pressure Vessel Code (BPVC)	Applies only to stainless steel columns
ASME BPE	Bioprocessing equipment	
UL 508a	UL standard for Safety for Industrial Control Panels	
EMEA/410/01, CPMP Note	All wetted polymer and elastomeric parts are animal origin-free or comply with the conditions in the standard, and are also classified according to USP Class VI, 21 CFR Part 177	

### **CE Marking**

The **CE** marking and the corresponding Declaration of Conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation.

The Declaration of conformity is valid only for systems that are marked with the **CE**-marking.



# Regulatory compliance of connected equipment

Any equipment connected to the AxiChrom 300-1000 columns and AxiChrom Master should meet the safety requirements of IEC EN 61010-1/ IEC 61010-1 or relevant harmonized standards. Within EU, connected equipment must be CE marked.

# 1.5 Intended use of the columns

AxiChrom columns are designed for process scale chromatographic separation of biomolecules such as proteins, peptides and oligonucleutides. AxiChrom columns can be operated at pressures up to 4 bar.

The columns must be used in accordance with the instructions and technical specifications in this manual and AxiChrom 300-1000 columns Operating Instructions to minimize the risk for injuries and damage to the columns or other equipment.

## **Approved operator**

The use of interactive guides in AxiChrom Master and in the Intelligent Packing wizard allows consistent performance with reproducible quality.

To use the column safely and correctly, the operator must have read, understood and be fully acquainted with AxiChrom 300-1000 columns User Manual and AxiChrom 300-1000 columns Operating Instructions. Improper operation of the column may result in property damage as well as personal injury.

# 1.6 Safety precautions

#### Introduction

Before installing, operating or maintaining the AxiChrom column, you must be aware of the hazards described in this manual. Follow the instructions provided to avoid personal injury or damage to the equipment.

The warnings and cautions in the user documentation shall in no way take precedence over more restrictive local regulations and policies.

For your personal safety it is important that you have a proper knowledge of the entire system that the column is part of. Study any complementary safety instructions and use appropriate personal protection equipment for the specific application and operation environment.

#### Risk assessment

AxiChrom columns have been designed and manufactured to provide a high level of personal safety. However, the residual risk is highly dependent on the application and environment in which the column is operated. In order to determine the safe operation of the equipment a risk assessment must be made. This risk assessment, in combination with local regulations and policies, will result in specific safety instructions for installation, operation and maintenance, use of proper personal protective equipment, or other arrangements that are needed to operate your process safely.

## **General precautions**



#### WARNING

The customer must ensure that all installation, maintenance, operation and inspection is carried out by qualified personnel who are adequately trained, understand and adhere to local regulations and the operating instructions, and have a thorough knowledge of AxiChrom 300-1000 columns and the entire process.



#### WARNING

AxiChrom columns must NOT be used for any other purposes than chromatographic separations. They must not be used as:

- Storage tanks for chemicals etc.
- General pressure vessels
- Fermentation vessels
- Gas storage tanks

The column must never contain air or gas under pressure.

# Using flammable liquids



#### WARNING

AxiChrom columns are not designed to handle flammable fluids. AxiChrom is not approved for work in a potentially explosive atmosphere, in areas classified as Zone 0 to Zone 2 according to IEC 60079-10 2002.

## **Personal protection**



#### WARNING

Always use protective glasses and other personal protective equipment appropriate to the current application, to ensure personal safety during operation.



#### WARNING

Disconnection of pressurized air supply tubes can cause loud noises. Ear protection is recommended.

Note:

No ear protection is required during normal operation. The noise from AxiChrom is low (below 55 dB).

# Installing and moving the columns and Master



#### WARNING

**Move transport crates.** Ensure that the forklift has capacity to safely lift the crate weight. Ensure that the crate is properly balanced so that it will not accidentally tip when moved.



#### WARNING

**Move column.** Ensure that the column's center of gravity is well balanced over the forklift's lifting arms, otherwise the column may tip off the forklift.



#### WARNING

All electrical installations must be performed by authorized personnel only.



#### **CAUTION**

Ensure that all tubing, hoses and cables are placed so that the risk for tripping accidents is minimized.



#### NOTICE

Disconnect all tubing, hoses and cables before moving the column.

# **System operation**



#### WARNING

The working pressure of the column should never exceed 4 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.



#### WARNING

Do not block access to the power switch. The power switch must always be easy to access.



#### CAUTION

Do not touch the motor on the column during operation since the motor might be hot and there is a risk of burning injury.



#### **CAUTION**

Do not stand on the column base to reach the top parts of the column (e.g. top inlet/outlet). Always use proper equipment for climbing and standing when reaching the top parts of the column.



#### **CAUTION**

Remove any spillage on the floor immediately to minimize the risk of slipping accidents.



#### CAUTION

Use a pressure gauge, pressure relief valve, rupture disc or other pressure safety equipment to ensure that the maximum operating pressure of the column is not exceeded.



#### NOTICE

Do not operate the column at temperatures outside the operating temperature range.



#### NOTICE

Ensure that the column and system is primed, that is, free from air, and that the inlet is open before the process run is started.



#### NOTICE

Do not use chemicals not listed in the Chemical Resistance information.

The wetted parts of AxiChrom may be damaged by chemicals not listed in the Chemical Resistance information. Contact your GE Healthcare representative before using chemicals that are not listed.



#### NOTICE

Ensure that any liquids used with the column is particle-free (down to  $1 \mu m$ ), as particles can block and damage the bed support.



#### NOTICE

Chlorides and low pH can cause corrosion on stainless steel. Rinse thoroughly with clean water after use.

Inspect the column regularly for signs of corrosive attacks, which may cause column damage if untreated. Note that the stainless steel bed supports are especially vulnerable to corrosion.



#### NOTICE

If there is air in the column, ensure that there is an unrestricted flow path for evacuation through an open valve before the adapter is moved or liquid is pumped into the column.



#### NOTICE

Ensure that the pressure does not exceed the operating pressure of the media packed in the column.



#### NOTICE

The acrylic column tube has limited resistance to organic solvents.

Refer to the Chemical resistance section for more information.

#### Maintenance



#### WARNING

Follow all safety instructions displayed in the AxiChrom Master when performing maintenance on the column.



#### WARNING

Ensure that no body parts are caught between the column tube assembly and the column frame when using the swing out function for maintenance work on the column. Due to the weight of the column tube assembly, it is not advisable to try to stop the momentum of the assembly by hand when it is set in motion.



#### WARNING

There must not be any residues of harmful substances left in the column during maintenance. Make sure that the column is properly cleaned before maintenance, and that cleaning is documented in the Decontamination Report.



#### NOTICE

Lifting the top unit with incorrectly placed lock blocks may damage the column.



#### NOTICE

Inspect all connections and tubing before use and replace any defective parts. Also inspect the column tube thoroughly before use to verify that there are no cracks or other visible signs of damage.



#### NOTICE

Perform a leakage test whenever any of the wetted parts have been re-fitted or changed.



#### NOTICE

Always wear latex gloves (or gloves of other suitable material) when handling the bed support to prevent grease from unprotected fingers coming into contact with the bed support.



#### NOTICE

To avoid spillage, do not open the bottom valve while the column is filled with liquid, unless there are hoses connected to the outlets.



#### NOTICE

Handle stainless steel parts, especially the bed supports, with care. Damage to surfaces may lead to corrosion.

# 1.7 Emergency stop procedures

#### **EMERGENCY STOP buttons**

AxiChrom Master is equipped with an **EMERGENCY STOP** button which can be used to stop the movement of the adapter in case of an emergency during operation of the column. When the **EMERGENCY STOP** button is pressed, the adapter movement stops. The process that is in progress in AxiChrom Master (for example an Intelligent Packing) will be aborted and the interface will display the **MAIN MENU**. The **EMERGENCY STOP** button locks in place until it is released manually.

The **EMERGENCY STOP** button is located on the AxiChrom Master. The **EMERGENCY STOP** label is yellow with black text and the button is red.







#### WARNING

Ensure that the **EMERGENCY STOP** button can always be reached while working with the column.



#### WARNING

The **EMERGENCY STOP** button will not shut off the electrical power to the motor of column. Use the power switch on the connector panel of AxiChrom Master to shut off the electrical power.

# EMERGENCY STOP buttons on ÄKTAprocess systems

The **EMERGENCY STOP** buttons on the system must be used in case of an emergency when the pumps have to be stopped. There are two **EMERGENCY STOP** buttons on an ÄKTAprocess system, one on each side, and only one needs to be pressed in order for the pumps to stop. Refer to the Operating instructions for more information about the emergency procedures for ÄKTAprocess.

# 1.8 Resetting the system after an emergency stop



#### WARNING

The column may still be pressurized when the process has been stopped using the **EMERGENCY STOP** button. Ensure that the pressure is released before disconnecting any tubing.

The **EMERGENCY STOP** button will abort the process that is currently in progress in Axi-Chrom Master, for example Intelligent Packing. The process has to be restarted from the beginning after the emergency has been resolved.

If the **EMERGENCY STOP** is pressed during the **MAINTENANCE** wizard or **MANUAL CONTROL** wizard, the **MAIN MENU** is displayed. When the assembly or disassembly process is launched again, the software returns to the same state as when the button was pressed.

The **EMERGENCY STOP** button is released by turning the button counter-clockwise. If the column was being packed, the column must be emptied using **MANUAL CONTROL** or **UNPACKING** in AxiChrom Master or the unpacking method in UNICORN before the Intelligent Packing wizard can be started again.

# 1.9 Disconnecting the power

The power to the AxiChrom column, AxiChrom Master and/or the system can also be shut off in an emergency by disconnecting the power supply using the system power switch. Note that disconnecting the power this way from an ÄKTAprocess system will cause valves to close immediately and not in a proper sequence. Ensure that the system and column is fully depressurized before disconnecting any tubing or removing components that are part of the flow path.



#### WARNING

It is essential that access to **EMERGENCY STOP** buttons, master power switch, system power switch and power cable connections are kept free. Do not block any of these items with other equipment, or position the column so that they become unreachable.



#### WARNING

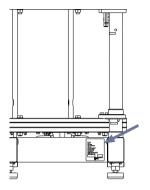
A disconnecting system power switch must be installed, such as a branch circuit protection and disconnect switch installed at your facility. The system power switch should be installed at the wall to cut the power to AxiChrom Master. Always use this power switch (located on the back on the AxiChrom Master unit) and the system power switch for safe disconnection of AxiChrom Master.

# 1.10 Labels

This section describes the identification labels, safety labels and labels concerning hazardous substances that are attached to the AxiChrom columns and AxiChrom Master.

# Column label

# Label placement



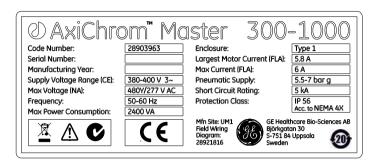
# Example label



Label text	Meaning
Dimension	Dimensions of the column
Serial number	Serial number for the individual column
Year of manufacturing	Year when the column was manufactured
Tare mass	Weight of the empty column
Operating temperature TS	Operating temperature range
Max column volume V	Maximum column bed volume
Design pressure PS	Design pressure for the column
Test pressure PT	Pressure at which the column has been tested in manufacturing
Date	Date of test
Maximum filling mass	Maximum weight of column contents (media and eluent)
PED fluid group / cat.	Pressure Equipment Directive fluid group / category

## **AxiChrom Master label**

The illustration below shows an example of an AxiChrom Master label.



Label text	Meaning
Code Number	Product code number for AxiChrom Master
Serial Number	Serial number for the individual AxiChrom Master
Manufacturing Year	Year when the AxiChrom Master was manufactured
Supply Voltage Range (CE)	Permissible supply voltage range (Europe)
Max Voltage (NA)	Maximum permissible supply voltage (North America)
Frequency	Supply voltage frequency
Max Power Consumption	Maximum power consumption
Enclosure	Enclosure rating according to EN 61326-1
Largest Motor Current (FLA)	Highest current used by the motor (Full Load Ampere)
Max Current	Highest current used by the AxiChrom Master
Pneumatic Supply	Required pressure for pressurized air supply
Short Circuit Rating	Maximum tolerated short circuit current
Protection Class	Protection class rating according to EN60529

# Symbols used in safety labels

Label	Meaning
$\triangle$	<b>Warning!</b> Read the user documentation before using the system. Do not open any covers or replace parts unless specifically stated in the user documentation.
C	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
CE	The system complies with applicable European directives.

# Labels concerning hazardous substances

Label	Meaning
X	This symbol indicates that waste electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning decommissioning of equipment.
<b>@</b>	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronics.

# 2 Specifications and characteristics

# 2.1 Principles of column function

# **Intelligent packing**

Intelligent Packing uses interactive guides to ensure an optimized compression of the bed. Intelligent Packing will pack the column with minimal manual intervention to ensure reproducible results.

Intelligent Packing is controlled from AxiChrom MasterMaster, either as a stand-alone unit or controlled in turn from an  $\ddot{A}KTA$ process $^{TM}$  instrument running UNICORN $^{TM}$  software.

## **Axial compression**

AxiChrom columns are packed using axial compression, achieved by an electric motor driving the adapter downwards through the column tube until the required packing is achieved.

# Filling the column

To fill the column, the adapter is raised to draw media slurry through the media valve inlet into the **process chamber**, i.e. the space between the adapter bed support and bottom bed support.

The correct slurry volume to draw into the column is calculated using

- the target bed height
- the slurry concentration
- a Packing Factor for optimal packing (defined later)
- the volume added by the tube leading from the slurry tank to the column.

# Packing the column

To pack the column, the adapter moves downwards, pushing down the slurry and forcing the liquid out through the bottom bed support and further out through the Bottom mobile phase. Media particles are retained by the bed support and a bed is formed. This is called the consolidation stage. The pressure is monitored in the closed Top mobile phase flow path. The pressure gradually increases while the bed is being consolidated. After the bed is fully consolidated the adapter will eventually meet with the newly formed bed. When this occurs, the pressure drops momentarily in the column. When the consolidated bed is detected, either by the decrease in pressure or manually, the operator is given an option to:

- Ignore if the detection was false. Result: The consolidation continues. If no detection occurs the adapter stops at the defined Low bed height limit.
- Confirm the detection. Result: If the final bed height is calculated to be within the
  selected bed height range with the defined Packing Factor, the adapter will continue
  to compress the bed. When the defined Packing Factor has been achieved, the operator ends the packing procedure. If the selected bed height range cannot be met
  the packing will be aborted. The compressed bed should be within the defined
  compression range within the set bed height range to assure optimal compression
  and performance of the packed bed.

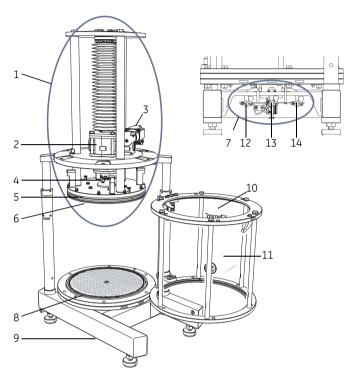
# Unpacking the media from the column

The unpacking of the media from the column is performed in three steps:

- expansion
- collapse
- slurry push out

The expansion is performed by lifting the bed by moving the adapter up at the same time as flow comes from the bottom bed support. When the bed has expanded the flow is reversed to collapse the bed by pushing it down. When the collapsed bed has fallen to the bottom of the column the Media valve opens and the slurry can be pushed out from the column by having a low upwards flow into the column and at the same time lower the adapter to the bottom position.

# 2.2 Overview of the column and parts



Part	Function	Part	Function
1	Top unit	8	Bottom bed support and distributor plate
2	Worm gear and bellows	9	Column stand
3	Servo motor	10	Process chamber
4	Top mobile phase inlet/outlet	11	Column tube
5	Adapter	12	Slurry inlet
6	Adapter bed support and distributor plate	13	Bottom mobile phase inlet/outlet
7	Media valve assembly	14	Rinse inlet

For information on column weights and dimensions, see *Appendix E Column and AxiChrom Master weights and dimensions*, on page 193.

## Inlet and outlet system

- The tubing connection at the Top mobile phase on the adapter is the only liquid connection at the top of the column.
- At the bottom the center inlet/outlet is the bottom mobile phase.
- The Slurry inlet is connected to the slurry tank from where the media slurry is drawn into the column. Inside the Media valve under the column, the Slurry inlet has a liquid connection with the Rinse inlet.
- The Rinse inlet is used to flush the Media valve, the Slurry inlet and the tube to the slurry tank free from residual media after filling and unpacking the column. When a filling process has ended, the Media valve closes and a pump connected to the Rinse inlet pumps the liquid through the Media valve, and then through the Slurry inlet to the slurry tank.
- The Slurry inlet and Rinse inlet have no connection to the Bottom mobile phase flowpath. When the Media valve is open, the Slurry inlet and Rinse inlet lead directly to the process chamber. Liquid in the mobile phases, on the other hand, has to flow through the bed supports to reach the process chamber.

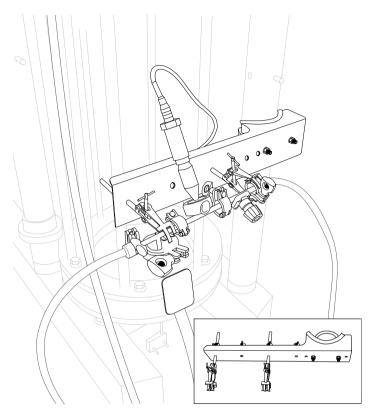
#### **Connection flanges**

- Tri-clamp 25 is used for the 300, 400, 450 and 600 columns.
- Tri-clamp 50 is used for the 800 and 1000 columns.

#### Valve holder (accessory)

The valve holder accessory is useful for fixing components to the column assembly, particularly when a number of serially connected valves and sensors are used. The valve holder is clamped on the front stand tube with a U-rod and holds the components with 3-pronged clamps. One valve holder carries two clamps. Different sizes of U-rod are used for AxiChrom 300-600 columns and AxiChrom 800-1000 columns respectively.

# 2.2 Overview of the column and parts



Valve holder accessory (inset) and mounted with valves and sensors on front stand tube.

#### **AxiChrom Master**

AxiChrom Master is a self-contained operator console featuring interactive guides for work procedures such as packing, unpacking and maintenance. The user interface is a touch screen panel.



The interactive firmware guides the user and reduces the risk of making mistakes. The operator has control over the workflow, and can use manual control for adapter movement and open or close the Media valve.

One AxiChrom Master unit can be used to control up to ten columns (one at a time).

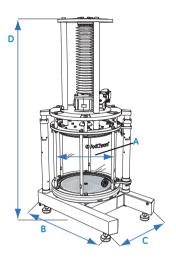
**Note:** Always use the same AxiChrom Master to control a given column. The motor will be recalibrated automatically if you change to a different Master.

#### **AxiChrom Master specifications**

Parameter	Value
Weight	Approximately 73 kg (161 lbs)
Length × width × height	670 × 590 × 1090 mm
Material	Stainless steel, ASTM 316 and ASTM316L (EN 1.4401/1.4436 and EN 1.4404/1.4432/1.4435)

# 2.3 Primary specifications

# 2.3.1 Weights, volumes and related dimensions



Column	300	400	450	600	800	1000
Tube inner diameter (A) (mm)	300	400	450	600	800	1000
Footprint (B×C) (mm)	1110 × 520	1110 × 600	1110 × 620	1180 × 780	1470 × 1080	1720 × 1300
Safety zone (mm)	3200 × 2600	3200 × 2600	3200 × 2700	3200 × 2800	3500 × 3100	3800 × 3300
Column cross section (cm <sup>2</sup> )	707	1257	1590	2827	5027	7854
Max bed volume, short/long tube (liters)	21/35	36/63	48/80	85/141	151/251	236/393
Weight of empty column, short/long column, stainless steel bed support (kg)	420/440	460/480	710/760	835/900	2150/2240	2560/2680
Weight of empty column, short/long column, plastic bed support (kg)	414/434	451/471	699/749	818/883	2122/2212	2517/2637

# 2.3.2 Heights in different operating states

**Note:** The letters in parentheses refer to the image on page 32. All dimensions are given in millimeters.

Column	Tube	300	400	450	600	800	1000
Max height <sup>1</sup> (D)	Short <sup>2</sup>	22	00	2230	2340	2630	2650
	Long <sup>3</sup>	27	20	2750	2860	3150	3170
Height (D) when adapter is at max bed	Short	17	40	1760	1870	2160	2170
height	Long	22	00	2220	2330	2620	2630
Min height (delivery height)	Short	1450	1460	1480	1590	1880	1890
	Long	1710	1720	1740	1850	2140	2150
Max slurry filling height	ax slurry filling height Short 2040		40	2060	2185	2335	2490
	Long	25	55	2580	2700	2850	3005
Max operating height (D) during priming	Short	2060		2080	2190	2480	2490
	Long	25	80	2600	2710	3000	3010
Max adapter stroke height for filling	Short	57		570		578	
	Long	8.		30		838	

- Refers to the height (height of casters excluded) of the column when the adapter is in its highest position, for example for working and service.
- 2 Bed height 100 to 300 mm
- 3 Bed height 100 to 500 mm

# 2.3.3 Operating conditions

Parameter	Value
Maximum operating pressure <sup>1</sup>	4 bar
Operating temperature <sup>2</sup>	2°C to +30°C
Operating pH <sup>3</sup>	1 to 14

- Refers to the maximum allowed pressure over the bed during normal chromatographic operation.
- See Section 3.9 Ambient temperature changes, on page 86.
- Due to corrosion, low pH values are unsuitable for solutions containing chlorides. See Section 2.5 Chemical resistance, on page 36.

# 2.3.4 Pressure and time out limits

# **Description**

The Master uses several different pressure limits during operation. When a limit is reached the adapter will stop (if moving) and an alarm text will be displayed. The limits are summarized in the table below.

Sequence		Limit
Manual	Low pressure	-0.5 bar
	High pressure	4 bar
Priming	Low pressure	-0.5 bar
	High pressure	4 bar
Filling	Low pressure	-0.30 bar
		The adapter stops automatically at the limit and continues again when pressure rises above - 0.25 bar
	High pressure	4 bar
Packing	Low pressure	-0.5 bar
	Overpacking	Overpacking safety stop 15 seconds after PF±0.03 is reached
	Bed detection	10 mbar pressure drop for more than 2 seconds (editable)
Unpacking	Low pressure	-0.5 bar
	Bed expansion	-0.30 bar
		The adapter stops automatically at the limit and continues again when pressure rises above 0 bar
	High pressure I	Regular unpacking for Capto™, Fast Flow and MabSelect™ media; Bead size > 50 µm: 0.5 bar
		The adapter stops automatically at the limit and continues again if the pressure falls below 0.2 bar
	High pressure II	Advanced unpacking for High Performance media; Bead size < 50 µm: 1.0 bar
		The adapter stops automatically at the limit and continues again if the pressure falls below 0.5 bar

Sequence		Limit
Maintenance	Low pressure	-0.5 bar
	High pressure	4 bar

# 2.4 Materials

## **Background information**

The materials used to manufacture AxiChrom columns have been chosen for their biological and chemical compatibility with the solvents used during operation and cleaning-in-place (CIP) procedures. The columns have also been designed to comply with the varying hygienic requirements at the different stages of development and production. Polymer materials in AxiChrom columns in contact with process liquids have been selected for their biological compatibility according to the United States Pharmacopeia (USP) Biological Reactivity Tests, *In vivo* and conform to USP class VI requirements, compliance with Code of Federal Regulations (CFR), Food and Drug Administration, Title 21, Part 177 and being animal free or complies with the conditions in the CPMP Note for Guidance (EMEA/410/01 Rev.2).

Column tubes for AxiChrom 300-1000 columns are available in acrylic plastic or stainless steel. Use and maintenance of AxiChrom columns with stainless steel column tubes differs from that for acrylic plastic column tubes in two major respects:

- Stainless steel column tubes are not transparent, so the bed cannot be observed directly. This affects packing and unpacking procedures.
- Stainless steel column tubes are manufactured in a single piece, with no removable tie rods, top or bottom flanges or corresponding O-rings. This does not affect normal operation or maintenance procedures but results in a spare parts and accessories list that differs from that of acrylic columns.

#### Parts list and materials

Component	Material	In contact with process stream
Adapter backing plate	Stainless steel ASTM 316 or ASTM S32205	No
Adapter seals and snap ring	UHMWPE (ultra high molecular weight polyethylene)	Yes

Component	Material	In contact with process stream
Bed support	Stainless steel ASTM 316L and ASTM S32205 or PE (polyethylene) or UHMWPE	Yes
Column tube	PMMA (polymethyl methacrylate) or Stainless steel ASTM 316L	Yes
Distributor	PP (polypropylene)	Yes
Dynamic seals	FFPM (full fluorinated propylene monomer) or UHMWPE	Yes
Media valve body	РР	Yes
Static seals	EPDM (ethylene propylene diene monomer)	Yes
Top mobile phase	РР	Yes
Bottom backing plate	Stainless steel ASTM 316	No
Lid	Stainless steel ASTM 316	No
Stand	Stainless steel ASTM 316	No

# 2.5 Chemical resistance

AxiChrom columns are resistant to chemical agents used in protein recovery, including buffer solutions for adsorption, elution and washing, and to solutions effective in cleaning, sanitization and storage. *Table* lists chemicals that may or may not be used with AxiChrom columns. The concentrations listed are not normally exceeded during an operating cycle.



#### **CAUTION**

Do not use chemicals with temperatures above the specified limits.



#### NOTICE

Chlorides and low pH can cause corrosion on stainless steel. Rinse thoroughly with clean water after use.

Inspect the column regularly for signs of corrosive attacks, which may cause column damage if untreated. Note that the stainless steel bed supports are especially vulnerable to corrosion.

Note:

The information in Table has been collected from several published sources, not from individual tests on column components. It should be used only as a guide. The effect of chemicals will generally be more severe at higher temperatures. Note also that the combined effects of agents have not been taken into account in this table. The applicable chemical resistance depends on the configuration of the column and the associated materials of construction.

Chemical	Concen- tration <sup>1</sup>	Time/cycle restrictions	Comments	Operating temperature	CAS no. <sup>2</sup>
Acetic acid	25%	3 h	Cleaning-In-Place (CIP)	2°C to 30°C	64-19-7
Acetone	2%	1 h	Efficiency test	2°C to 30°C	67-64-1
Ammonium sulphate	2 M <sup>3</sup>	5 h	Adsorption	2°C to 30°C	7783-20-2
Benzyl alco- hol	2%	12 months	Storage	2°C to 30°C	100-51-6
Ethanol	20%	12 months and max. 0.5 bar	Storage	2°C to 30°C	64-17-5
Ethanol	70% <sup>4</sup>	3 h	CIP	2°C to 30°C	64-17-5
Ethanol/ acetic acid	20%/ 10%	3 h	CIP	2°C to 30°C	64-17-5/ 64-19-7
Guanidinium hydrochloride	6 M <sup>5</sup>	5 h	CIP	2°C to 30°C	50-01-1
Hydrochloric acid	$0.1  \text{M}$ $(\text{pH} = 1)^6$	1 h	CIP	2°C to 30°C	7647-01-0
Isopropanol	30% <sup>7</sup>	1 h	CIP	2°C to 30°C	67-63-0

#### 2.5. Chemical resistance

Chemical	Concen- tration <sup>1</sup>	Time/cycle restrictions	Comments	Operating temperature	CAS no. <sup>2</sup>
Phosphoric acid	5%	8 h	For passivation of stainless steel bed supports	2°C to 30°C	7664-38-2
Sodium chloride	0 to 3 M <sup>3, 6, 8</sup>	3 h	Purification, CIP	2°C to 30°C	7647-14-5
Sodium hydroxide	1 M (pH = 14)	24 h, room temp. to 30°C	CIP	2°C to 30°C	1310-73-2
Sodium hydroxide	0.01 M (pH = 12)	12 months	Storage	2°C to 30°C	1310-73-2
Sodium hydroxide/ ethanol	1 M/ 20%	3 h	CIP	2°C to 30°C	1310-73- 2/64-17-5
Sodium sulphate	1 M <sup>3</sup>	3 h	Adsorption	2°C to 30°C	7757-82-6
Urea	8 M <sup>3</sup>	5 h	Purification, CIP	2°C to 30°C	57-13-6
Commonly used aqueous buffers for chromato- graphic use	10 to 250 mM, pH 2 to 10	24 h	Equilibration, adsorption, elution	2°C to 30°C	

- When a concentration is given as a percentage, this is v/v.
- <sup>2</sup> CAS no.: Registration number assigned by the Chemical Abstract Services (CAS), American Chemical Society.
- <sup>3</sup> pH in these solutions depends on the pH of the buffer, which can vary between 3 and 13.
- 4 On stainless steel column parts only. Exposure of other column parts to ethanol at higher concentrations than 20% can damage the column.
- Not for use with columns containing wetted components of stainless steel.
- 6 pH below 4 for stainless steel is not recommended.
- Applies only to acrylic column tubes.
- 8 For columns containing wetted stainless steel components max 1.0 M NaCl is recommended. For additional important information, see Section 2.5.1 Resistance to sodium chloride, on page 39.

# Avoid using the following chemicals in AxiChrom columns:

- Extreme oxidizers
- Fluorine and halogenated compounds
- Chlorinated solvents (such as methylene chloride)
- Esters
- Aromatic hydrocarbons (such as toluene)
- Alcohols at concentrations higher than those specified in the table above
- Salt in combination with pH below 4. Always wash the column with at least five column volumes of pH-neutral solution (water) between and after use of salt and low pH buffers.

## 2.5.1 Resistance to sodium chloride

Depending on configuration, AxiChrom columns can be equipped with wetted components of stainless steel and must therefore be appropriately maintained when exposed to sodium chloride (NaCl) during chromatographic processes. Since the bed supports are made of stainless steel and have a high degree of resistance to corrosion, they are susceptible. The degree of susceptibility is largely due to conditions of use. Low pH in combination with NaCl, high temperature and surface damage all increase risk of corrosion. If a stainless steel surface is damaged (scratches, impact marks etc.) is has to be cleaned and passivated prior to taken into operation. Your GE Healthcare representative can provide guidance on suitable cleaning and passivation procedures if required.

To clean the column from chloride ions, it is recommended that a water rinse of at least five column volumes is used. Water is the preferred rinse solution due to the solubility properties of sodium chloride in water compared to other possible rinsing solutions, for example ethanol solutions.

Columns should be stored with solutions free from chloride ions.

# 3 Getting started

# 3.1 Unpacking the crates and cleaning a new column

# Unpacking the crates

The following manuals contain information about how to unpack a new column:

- Read Me First contains specific instructions on how to unpack the column and Axi-Chrom Master from their crates. The instructions are attached to the outside of the packing crates upon delivery. Refer to those instructions when unpacking the products from their crates.
- AxiChrom columns Site Preparation Guide provides guidelines and requirements regarding how to place the column.

Note:

The column is delivered partially filled with 20% isopropyl alcohol/ethanol for conservation. Make sure that this solution is washed out according to the instructions below



### WARNING

Only personnel with appropriate training may move and unpack the delivery crates. All movements and unpacking must be performed in adherence with local regulations.

Even if the instructions in the Safety Instructions and User Manual are followed, it is the customers' responsibility to guarantee the safety of the personnel working with AxiChrom columns.



## WARNING

The column packing crate is marked with the center of gravity. Ensure that the crate is properly balanced so that it will not accidentally tip when moved.

# Cleaning a new column

The column is delivered partially filled with 20% (v/v) ethanol (storage solution) for conservation. The column must be unpacked from the crate and washed as soon as possible after delivery, preferably within 3 months. Please make sure that the column is in correct condition and free from damage according to the instructions in Read Me First.



#### WARNING

Always use protective glasses and other personal protective equipment appropriate to the current application, to ensure personal safety during operation.

If	Then
The column is to be used soon	Follow the instructions in Section 3.8 Cleaning the column, on page 85
The column is to be used at a later time (within 3 months from delivery)	Unpack the column from the crate, check it and clean it according to the instructions below  Note:  If the column is not cleaned, there is a substantial risk of
	mold and corrosion attacks.

# Cleaning procedure

- 1 Remove the tri-clamp blind cap from the Bottom mobile phase inlet/outlet, note that the column may be under slight pressure.
- 2 Connect a pump to the Bottom mobile phase inlet/outlet.
  - Ensure that the tube from the pump is primed, that is completely filled with liquid, before it is connected to the Bottom mobile phase inlet/outlet.
  - The pump must be switched off during installation, so that the column does not get pressurized.
- 3 Remove the tri-clamp blind cap from the tube at the Top mobile phase and connect a tube that leads to waste.
- 4 Flush the column with at least 6 column volumes (calculated from an adapter position of 1 to 2 cm at delivery) of 20% ethanol.
- 5 Prepare the column for storage, according to AxiChrom Operating Instructions.



#### NOTICE

If the column is filled and should be moved, plug the Top mobile phase and Bottom mobile phase inlets with tri-clamp blind caps, diaphragms or other suitable valves.

Note:

The tube installed on the Top mobile phase at delivery is only intended for storage solution conditioning and should be discarded once the column is installed. The maximum operating pressure of the tube is 1 bar.

# 3.2 Placing the column and Master

# Placing the column



#### NOTICE

Relieve the pressure from the column legs with a pallet jack or a lever while adjusting the legs, or else the screw threads may get damaged.

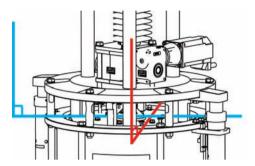


### NOTICE

Make sure that the column stands exactly level after it has been moved. If the column is not level, this can impact column operations such as packing and the resulting bed efficiency.

- 1 Use a pallet jack or the lever (used for assembling wheel kits) to relieve the pressure from the column legs while adjusting them.
- 2 Carefully check for wobble. Make sure there is even load on all 4 feet.

A spirit level placed diagonally on top of the column will show when the column stands horizontal. Ensure that the column is level in all directions by measuring with the spirit level on different places of the column.

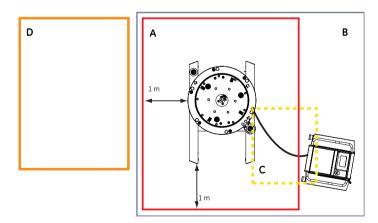


When the column feet have been adjusted, tighten the lock nuts on the feet using a tightening torque of 70 Nm.

# **Placing Master**

Place AxiChrom Master outside the AxiChrom column safety zone (A).

# Description of the safety zones



Zone	Description
Α	AxiChrom column Safety zone (red)
В	Service access area (blue)
С	Off limit zone (yellow) which is an area where cables and tubes are located
D	Approximate location (orange) of a connected ÄKTAprocess system (optional). Note that there will be more off limit zones with cables and tubing when an ÄKTAprocess system is connected.

## Safety zone dimensions

Column size, mm	300	400	450	600	800	1000
Safety zone length (A), mm		3	200		3500	3800
Safety zone width (B), mm	260	0	2700	2800	3100	3300



## WARNING

To avoid injury, no persons are allowed to be present inside the safety zone when the column adapter is in motion during maintenance procedures, see figure on page 43. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.



### WARNING

To avoid injury, no persons are allowed to be present inside the yellow zone: the area around cables and tubing running between the column to the Master, see image on page 43.



#### WARNING

Move Master so that it is always close to where the work is performed. The **EMERGENCY STOP** button on Master should always be in reach.

# Moving the column without wheels

All columns can be ordered with wheels as accessories. Columns without wheels must be moved with a forklift, pallet lift, or lifted by a hoist or crane attached to the adapter rods. A forklift or pallet lift with a distance of about 670 mm between the outer edges of the forks is recommended.



#### WARNING

Ensure that the forklift has capacity to safely lift the column weight. The column weight is shown on the rating plate.



### WARNING

When lifting with forklifts, pallet lifters or similar devices, the column must be lifted from the long sides. Do not try to lift the column from the back (the short side).



## WARNING

Ensure that the column's center of gravity is well balanced over the lifting forks, otherwise the column may tip off the forklift.



#### NOTICE

When moving the column using a forklift or pallet lifter, make sure that the media valve under the bottom of the column is not damaged. Close the media valve so that it protrudes less.

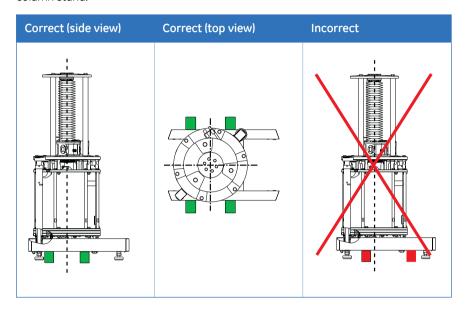


#### NOTICE

If the column is to be moved when it is filled, plug the top and bottom mobile phase inlets with tri-clamp blind caps, diaphragms or other suitable valves.

# Forklift placing

Always place the forks of the forklift or pallet lifter under the bottom of the column. Make sure that the forks are properly placed in relation to the center of gravity label on the column stand.



# 3.3 Wheel kits



### WARNING

Columns in swing-out or any kind of maintenance mode must not be moved. This may cause personal injury or damage to the column.



### **CAUTION**

To avoid accidental collisions, use caution when moving columns equipped with wheels by hand. Two or more people may be required to move the column safely.



#### NOTICE

To avoid damage to the wheels and possible tipping when the column or AxiChrom Master is moved, ensure that the wheels do not run into objects (for example thresholds or tubing).



### NOTICE

Columns equipped with casters must be levelled properly with the casters removed before the column is used.

# Wheel kits on AxiChrom 300-600 columns

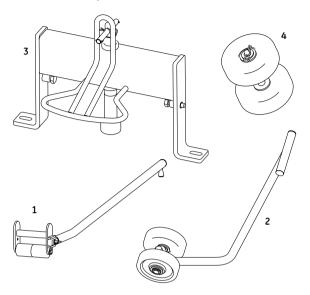
The wheels for 300-600 columns are delivered in a kit containing rear wheels, lever, trolley bar and trolley. Different column sizes use different wheel kits.



### NOTICE

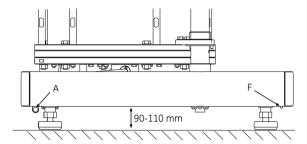
While transporting a 300-600 column, do not pull the handle upward, as the trolley knob may fall out of its position. Lifting the handle will not work as a brake.

# Wheel kit components



Part	Function
1	Lever
2	Trolley
3	Trolley bar
4	Rear wheels

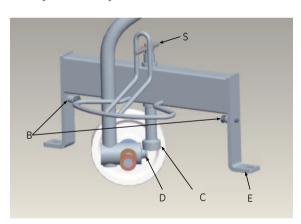
# Wheel and trolley fittings



Part	Function
Α	Hole for fitting rear wheels
F	Pins for locating trolley bar

**Note:** To be able to assemble or disassemble the wheels, the distance between the floor and the column stand must be in the range 90-110 mm.

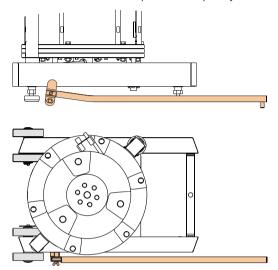
# Trolley and trolley bar



Part	Function
В	Bolts
С	Height adjusting screw
D	Trolley knob
Е	Slots
S	Safety pin

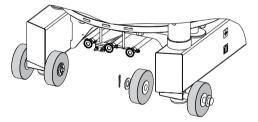
# Assembling the wheels on AxiChrom 300-600 columns

1 Place the lever close to one of the rear feet and lift the column by lowering the lever arm. Screw the foot pads in completely.



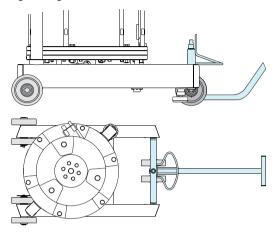
If needed for work in restricted spaces, the lever can be mirrored by changing the attachment of the lever arm.

Fit the rear wheels in the hole in the column stand (A, Wheel and trolley fittings, on page 49). Ensure that the washer and safety pin are correctly placed, to secure the rear wheels.



- 3 Lower the column by lifting the lever arm. Ensure that the column stands firmly on the wheels before removing the lever.
- Fit the second pair of rear wheels on the other rear foot by repeating steps 1 to 3 above.

Attach the trolley bar to the front of the column stand. Make sure that the pins underneath the stand (**F**, *Wheel and trolley fittings, on page 49* are properly fitted into the slots **E** of the trolley bar (*Trolley and trolley bar, on page 49*), before tightening the bolts **B** to the column stand.



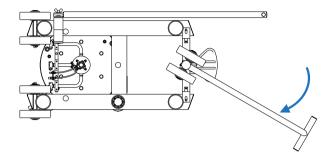
- Place the trolley knob **D** (*Trolley and trolley bar, on page 49*) vertically under the adjusting screw **C**, and adjust the height adjusting screw so it rests on the trolley knob.
- 7 When the height adjusting screw is adjusted, secure it with the safety pin **S**.
- 8 Lift the front of the column by lowering the handle on the trolley. Turn the trolley so it faces forward.
- 9 Ensure that the column fully rests upon the wheels. Screw the foot pads in completely.

The column can now be moved

## Disassembling the wheels on AxiChrom 300-600 columns

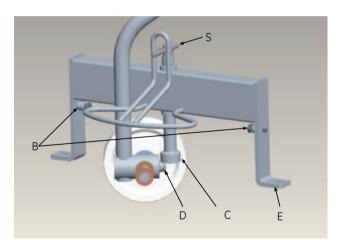
Adjust all feet until they have the same distance between the floor and stand, 90 to 110 mm.

2 Turn the trolley to one of the sides as the following image shows.



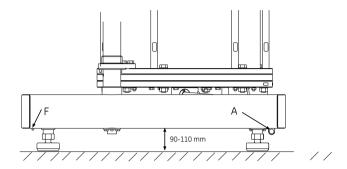
Lower the front of the column, by raising the handle on the trolley. The handle should now stand vertically.

If necessary, remove the safety pin and loosen the height adjusting screw to be able to relieve the weight from the trolley knob **D**. Remove the trolley.



- 4 Unscrew the bolts **B** and remove the trolley bar from the front of the column stand.
- Place the lever close to one of the rear feet and wheels, and lift the column by lowering the lever arm. If needed in narrow spaces, the lever can be mirrored by changing the attachment of the lever arm.

Remove the washer and safety pin from the wheels, and disassemble the wheels from the hole **A** in the column stand.



- 7 Lower the column by lifting the lever arm. Remove the lever.
- 8 Repeat steps 4 to 6 on the other rear foot.
- 9 Ensure that the column stands firmly on all feet.
- 10 Ensure that the column is level, see *Placing the column, on page 42*.

# Wheel kits on AxiChrom 800 and 1000 columns

The wheels for 800 and 1000 columns are delivered in a kit containing a jack, trolley bar, bracket and tools. Different column sizes use different wheel kits. All equipment needed for fitting and removing the wheels and trolley bar is stored in the box on the trolley.

# Assembling the wheels on AxiChrom 800 and 1000 columns

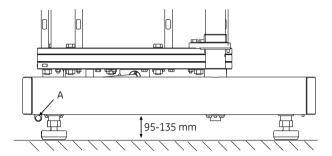
**Note:** To be able to use the jack when assembling/disassembling the wheels, the

distance between the floor and the column stand must be between 95-135  $\,$ 

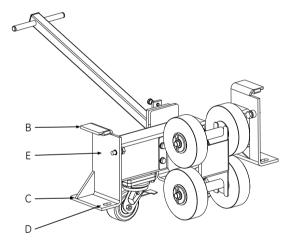
mm for AxiChrom 800 and 1000 columns.

**Note:** The distance between the floor and the column stand is 122 mm when the

wheels are assembled.



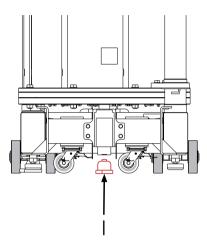
- 1 Mount the wheels (2) on the underside of the wheel kit beam. Mount the trolley handle.
- Use the handle on the trolley bar to steer it in between the front feet of the column. The top **B** of the trolley bar should hang on the column stand when positioned.



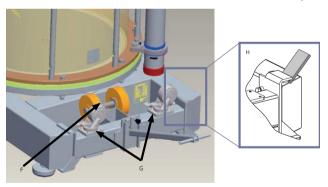
Part	Function
В	Trolley bar top
С	Lower part
D	Slot
Е	Screw

Remove the rear wheels from the wheel kit frame.

Place the jack under the frame at position  $\mathbf{I}$ , and use the jack to lever the front of the column so that the wheel kit can be assembled and tightened with the 16 mm wrench underneath the trolley bar  $\mathbf{G}$ .



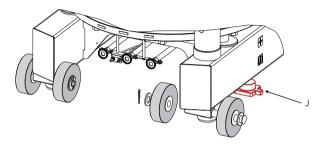
4 Place the bracket between the column stand and the trolley bar, as in **H**.



Steer the wheel kit with the trolley handle and lift up until part  ${\bf C}$  reaches the lower surface of the column stand. Make sure that the pin underneath the stand fits inside the slot  ${\bf D}$  of the trolley bar, then tighten the screw  ${\bf E}$  in the image in step 1. Make sure that both screws  ${\bf E}$  are in the correct position.

Remove the handle from the trolley bar. The handle is only a help when putting the trolley bar into position and should not be used when moving the column.

- 6 Lower the column front again and remove the jack. The column should now stand on the front wheels of the trolley bar, and the front feet should not touch the ground. Both wheels should be locked facing the direction of the frame legs before continuing to the next step.
- Place the jack **J** under the frame at the reinforced pad towards the rear wheel and lift.

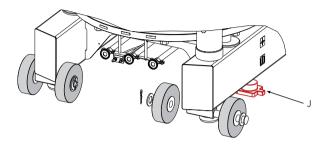


- Fit the rear wheels in the hole **A** in the column stand (see illustration on page 54. Ensure that the washer and safety pin are correctly placed, to secure the rear wheels.
- 9 Lower the column again and remove the jack. Make sure that the front feet do not touch the ground.
- 10 Fit the second pair of rear wheels on the other side of the column by repeating steps 7 to 9.
- Ensure that the column rests fully on the wheels.
  The column can now be moved using a MasterMover connected to the fitting on the front of the trolley bar.

# Disassembling the wheels on AxiChrom 800 and 1000 columns

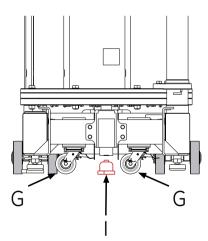
Adjust all feet until they have the same distance between the floor and stand, 95-135 mm

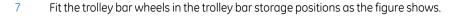
Place the jack J under the frame at the reinforced pad towards the rear wheel and lift.

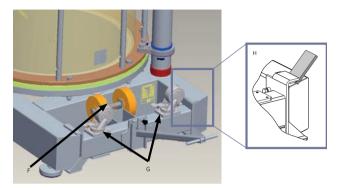


Lever the back of the column using the jack.

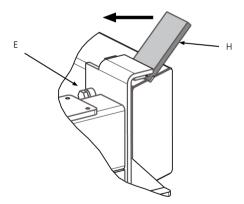
- 3 Remove the rear wheels from the hole on the column stand.
- 4 Lower the column again and remove the jack.
- Remove the second pair of rear wheels on the other side of the column by repeating steps 1 to 4.
- Place the jack at position I in the figure. Use the jack to lever the front of the column so that the wheel kit can be removed from underneath the trolley bar **G**. Lower the column front again and remove the jack. The column should now stand on its front feet.







- 8 Fit the rear wheels in the trolley bar storage positions **F**.
- 9 Attach the handle to the trolley bar.
- 10 Unscrew the screw **E** on one of the trolley bar sides and insert the bracket **H** between the trolley bar and the column stand. Use the bracket to carefully lower the trolley bar down until the trolley bar rests upon the column stand.

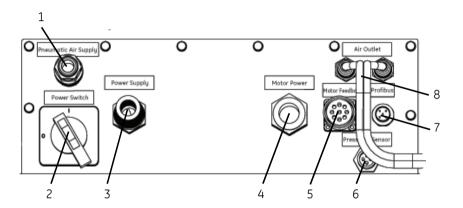


- 11 Repeat step 10 to release the other side of the trolley bar.
- 12 Use the handle to retract the trolley bar from the column stand.
- 13 Ensure that the column stands firmly on all feet.
- Ensure that the column is level, see *Placing the column, on page 42*.

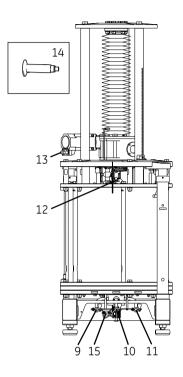
# 3.4 Connections

# Connections on Master and column

The illustrations below show the AxiChrom Master connector panel and the connection points on a column.



Part	Description	Part	Description
1	Pneumatic air supply	5	Motor feedback
2	Power switch	6	Pressure sensor connector
3	Power supply cable	7	Profibus signal cable connector
4	Motor power cable	8	Two air outlet connectors



Part	Description
9	Slurry inlet
10	Bottom mobile phase inlet/outlet
11	Rinse inlet
12	Top mobile phase inlet/outlet
13	Motor power and motor feedback
14	Pressure sensor (PIS_119, mounted on hose to system)
15	Pneumatic inlets with 2 connectors

# Connect column, Master, and external system

The table below shows how to connect the column, Master and an external system. Numbers in parentheses refer to the illustration *Connections on Master and column, on page 59*.



### **CAUTION**

Use a pressure gauge, pressure relief valve, rupture disc or other pressure safety equipment to ensure that the maximum operating pressure of the column is not exceeded.

From	То
Pneumatic air supply (1) on the Master	Wall socket air outlet (5.5-7 bar)
Motor power cable (4) on the Master	Motor power (the right connector) on the column (13)
Motor feedback (5) on the Master	<b>Motor feedback</b> (the left connector) (13) on the column
<b>Pressure sensor connector</b> (6) on the Master	Pressure sensor mounted on system (14)
<b>Profibus signal cable</b> (7) on the Master	Profibus connection on an ÄKTAprocess system
<b>Two air outlet connectors</b> (8) on the Master	<b>Pneumatic inlets with 2 valves</b> (15) on the column
Slurry inlet (9) on the column	Slurry tank
Bottom mobile phase (10) on the column	<b>Bottom mobile phase</b> on a system ( <b>Column1 bottom valve</b> on the ÄKTAprocess system)
Rinse inlet (11) on the column	A system ( <b>CIP2 Inlet</b> on the ÄKTAprocess system)
<b>Top mobile phase</b> (12) on the column	<b>Mobile phase</b> on a system ( <b>CIP1 Inlet</b> on the ÄKTAprocess system)
<b>Protection ground cable</b> on the column stand	<b>Ground</b> (See Grounding the column, on page 65)
Power supply cable (3) on the Master	Power supply connector (380-400 VAC, 50-60 Hz) with protective ground (The AxiChrom Master is delivered with CE or UL approved cables.)

# Recommended mobile phase tubing inner diameters

Note:

- All dimensions are given in millimeters.
- A dash (-) means that the combination is not compatible with Intelligent Packing.
- TC25 connectors do not have the same inner diameters as the tubing. See Tubings, on page 187 for details.

Table 3.1: Tubing inner diameters recommended for top and bottom mobile phase connections for different column inner diameters.

ÄKTAprocess dimension	300	400	450	600	800	1000
6 mm PP	6.4	6.4	6.4		-	-
OHIHIFF	9.4	9.4	9.4	_		
3/8" SS	6.4	6.4	6.4			
	9.4	9.4	9.4			-
10 mm PP	6.4	6.4	6.4	9.4		
	9.4	9.4	9.4	12.7	_	-
	12.7	12.7	12.7	19.1		
1/2" SS	6.4	6.4	6.4	9.4		
	9.4	9.4	9.4	12.7	_	-
	12.7	12.7	12.7	19.1		
1" PP and SS		-	-	9.4	25.4	25.4
	-			12.7	34.7	34.7
				19.1		

Use narrower tubing for packing gel filtration or polishing media. Use the widest tubing compatible with the system for high flow media such as Capto.

# Power requirements and connections

The general requirements are:

Requirement	Value
Supply voltage	380-400 VAC
Nominal current	10-15/16 A NTD (Non-Time Delay) (minmax.)
Frequency	50 - 60 Hz
Max voltage (North America)	480 Y/277 VAC
Max current	6 A
Max power consumption	2400 VA
Short circuit rating	5 kA

Note:

Detailed requirements may vary for different column configurations. Refer to the column documentation for the applicable requirements for your specific column and AxiChrom Master.

The power cables for both the AxiChrom Master and for the adapter control motor are delivered attached to the AxiChrom Master.

The system shall be connected to a 400 V supply voltage system or a 480 V supply voltage system with a maximum voltage of 277 V between phase and ground. A suitable connector has to be selected and assembled on the AxiChrom Master by the customer to comply with the supply voltage system and local regulations.

The **Motor Power** cable for the adapter control motor is permanently installed on the AxiChrom Master. The motor feedback communication cable is connected to **Motor Feedback**. Both these cables must be connected to the control motor before AxiChrom Master is connected to the power outlet.

## Color coding of cable conductors

Conductor	Color
Protective ground (earth)	Green/yellow
Live 1	No 1 or Black
Live 2	No 2 or Brown
Live 3	No 3 or Grey

The black, brown and grey conductors may be connected to any of Live 1, 2 and 3. The phase connection is detected automatically.



#### WARNING

Ensure that the **EMERGENCY STOP** button can always be reached while working with the column.



### WARNING

Electrical installations must be performed by authorized personnel only.



#### WARNING

Only authorized personnel are allowed to open the cabinet of Axi-Chrom Master. There are no user-serviceable parts inside the cabinet.



### WARNING

Always connect AxiChrom Master to a power supply with protective grounding.



#### WARNING

Connection to wrong power supply voltage may cause injury to personnel and damage to the system.



### WARNING

The power cables must be replaced only by cables of the same type or equivalent, fitted with the same type of connectors.

Power cables and connectors must be replaced or repaired by properly trained personnel, authorized by GE Healthcare.



## WARNING

The power connection must be easily accessible to enable the user to disconnect the power in case of an emergency.



#### NOTICE

Connect the communication cable and power cable for the adapter control motor on the column to AxiChrom Master before AxiChrom Master is connected to power.

**Note:** This is a class A product, input power >1 kW, intended for professional use. In

a domestic environment it may cause radio interference, in which case the

user might be required to take appropriate measures.

**Note:** This equipment complies with FCC part 15 (2004): Radio frequency device subpart B: Unintentional radiators, Class A. Operation is subject to the following two conditions:

1 This equipment may not cause harmful interference.

2 This equipment must accept any interference received, including interference that may cause undesired operation.

# Grounding the column

The column should always be connected to ground using a ground cable kit. Use the connection point on the inside of the column stand, to ground the column to a suitable grounding point.

The illustration below shows the connection point for protective ground cable on the column frame.

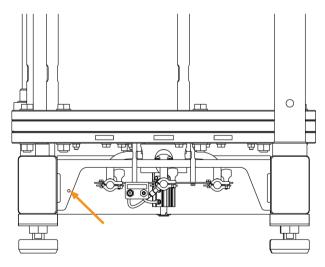


Figure 3.1: Connection point on the column frame for protective ground cable.

# **Compressed air requirements**

It is important for personal safety and safe operation to use the correct pressure and quality of compressed air for the pneumatic valve control. The basic requirements are:

- Free of oil and particles
- -30°C dew point
- 5.5 to 7 bar

The pneumatic air supply connections on AxiChrom Master are illustrated in *Connections* on Master and column, on page 59.

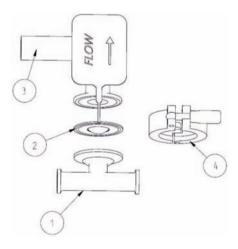
# 3.5 Rupture discs

To secure equipment and personal safety, rupture discs are available as accessories.

**Note:** Installation of rupture discs will change the maximum operating pressure for the column from 4 to 3.8 bar (g) due to rupture disc characteristics.

For column sizes 300-600 there is a special T-junction provided for use with rupture discs. Use this junction only with rupture discs recommended by GE Healthcare.

For column sizes 800-1000 use a standard 1" T-junction (part no. 28938170).



Part	Description	Part	Description
1	T-junction	3	Vent
2	Rupture disc	4	Clamp

Figure 3.2: Rupture disc assembly

## Installing rupture discs



#### NOTICE

Rupture discs should be installed at both mobile phases.

To install rupture discs, do as follows:

- 1 Connect the T-junctions to the column mobile phases according to Section 3.6 Column and system setup, on page 68.
- 2 Choose appropriate hose or pipe with a TC50 ferrule as vent.



#### CAUTION

Do not place rupture disc vent towards an area where it would endanger personnel.

- 3 Verify and ensure that all ferrule and clamp working surfaces are clean, dry, and free of nicks, dents, gouges, and wear.
- 4 Inspect gasket position and ensure that gasket ID is centered on the dome of the disc, then place the rupture disc into the ferrules with the flow arrow on the rupture disc tag pointing in the same direction as the desired vented flow after rupture.



### NOTICE

Always handle rupture discs with extreme caution.

- 5 Install the clamp around the ferrules so that the gap between the two clamp halves is centered on the rupture disc tag.
- 6 Hand-tighten the wing nut until the clamp is fully engaged with the ferrules and the rupture disc is securely in position. The nut will turn freely until it becomes snug with the clamp. Tighten another 1 to 1.25 turns.
- 7 Perform column and system setup according to the relevant instructions in Section 3.6 Column and system setup, on page 68.



### **CAUTION**

Double check the orientation of the rupture disc. Verify that the flow arrows on the disc tags are pointed in the same direction as the desired vented flow after rupture.

# 3.6 Column and system setup

## In this section

This section describes how to connect the AxiChrom column to tanks, pumps and for example an ÄKTAprocess system, for priming, packing and unpacking sequences in the AxiChrom Master.

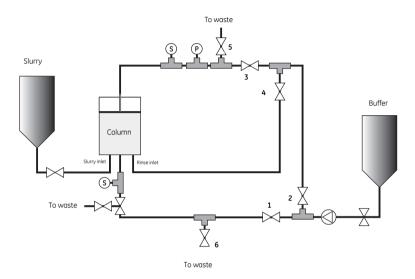


## WARNING

A pressure relief valve, rupture disc or a similar over pressure protector must be installed at both of the mobile phases, before the column is used. Pressure relief valves and rupture discs can be ordered as accessories from GE Healthcare. Ensure that any liquid that is ejected by the over pressure protection equipment is connected to a waste outlet.

# **Manual configuration**

The following manual setup makes it possible to perform all processes available in the AxiChrom Master: **PRIMING**, **INTELLIGENT PACKING** and **UNPACKING**. The setup comprises two way valves and shut-off valves to utilize different flow paths for the different procedures.



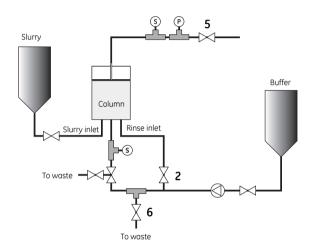
Symbol	Function
<u>\$</u>	Pressure relief valve, rupture disc or similar
<b>\$</b>	T-piece
*	2-way membrane valve/shutoff valve
<b>→</b>	2-way membrane valve/shutoff valve with bleed, can be replaced with two 2-way membrane valves and a T-piece

# Possible flowpaths for different column operations

Process	Flow path alternatives (from > to)
Priming	Pump > bottom mobile phase > waste (valve 5)
	Column > bottom mobile phase > waste (valve 6)
Packing	Column > bottom mobile phase > waste (valve 6)
Rinse	pump > valve 2 > valve 4 > rinse > slurry tank
Unpacking "upflow"	pump > bottom mobile phase > top mobile phase > waste (valve 6)
Unpacking "me- dia push out and down flow"	pump > bottom mobile phase > top mobile phase > closed > media valve open to slurry tank
	pump > valve 2 > top mobile phase > bottom mobile phase > waste (valve 6)

# Manual configuration: minimal setup

The following manual setup makes it possible to perform the *PRIMING* and *INTELLIGENT PACKING* procedures available in the AxiChrom Master. The setup comprises two way valves and shut-off valves to utilize different flow paths for the different procedures. For symbol explanations, see the table on page 69.

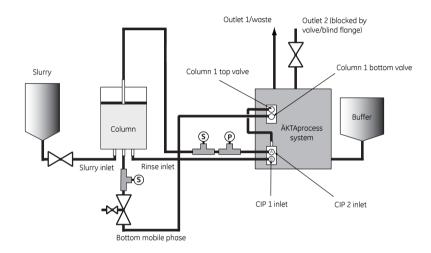


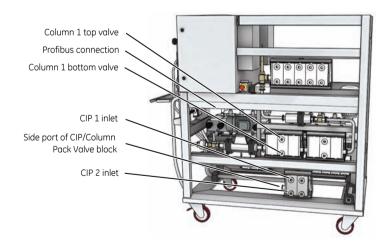
# Possible flowpaths for different column operations

Process	Flow path alternatives (from > to)
Priming	Pump > bottom mobile phase > top mobile phase > waste (valve 5)
Filling	Column > bottom mobile phase > waste (valve 6)
Packing	Column > bottom mobile phase > waste (valve 6)
Rinse	Pump > valve 2 > rinse > slurry tank

# Automatic configuration connecting ÄKTAprocess

The following setup makes it possible to perform all procedures that are available in the Method wizard in UNICORN: *Priming, Intelligent Packing* and *Unpacking*. The setup is simplified by using ÄKTAprocess controlling the pump and valves for flow directions. For symbol explanations, see the table on page 69.





# Connecting to an ÄKTAprocess system

To simplify automated priming, column packing with HETP evaluation or column unpacking procedure, the AxiChrom column has to be connected to a ÄKTAprocess system (equipped with the optional CIP/Column Packing valve block) as described below. For connections on AxiChrom Master and column, see *Section 3.4 Connections*, on page 59.

#### Note:

- Use the Column1 valves If the ÄKTAprocess system is equipped with two column valve blocks.
- During priming a membrane valve should be attached to the top mobile phase.

#### **Procedure**

- 1 Connect a short piece of tubing of suitable length and the same inner diameter as the system, from Column1 top valve to Side port of the CIP/Column Pack valve block
- 2 Connect the AxiChrom Master pressure transmitter PIS 119 to INLET CIP1.
- 3 Connect a pressure relief valve or similar to **PIS\_119**.
- 4 Connect a tube of suitable length and inner diameter, from the column **Top mobile phase** to the pressure relief valve on AxiChrom Master pressure transmitter **PIS\_119**. For tube diameter recommendations see *Recommended mobile*phase tubing inner diameters, on page 62.
- 5 Connect a tube of suitable length and inner diameter from **CIP2** on the process system CIP/Column Pack valve block to the Rinse inlet on the column.
- Connect a tube of suitable length and inner diameter from **Bottom mobile**phase on the column to a pressure relief valve, rupture disc or similar and then to **Column1 bottom valve** on the process system.
- 7 Connect the profibus cable from the Profibus connection situated under the electrical cabinet of the ÄKTAprocess system to the Profibus connection on the AxiChrom Master, see Connecting AxiChrom Master to ÄKTAprocess via Profibus, on page 73.

# Connecting AxiChrom Master to ÄKTAprocess via Profibus

#### Connect the Profibus cable

1 Power down the ÄKTAprocess and Master units.

2



#### CAUTION

Make sure that the ÄKTAprocess unit is powered off before the following step is performed.

Remove the Profibus termination plug from the ÄKTAprocess bulkhead connector.

- Connect the Profibus cable to the ÄKTAprocess bulkhead connector. Make sure that the cable barrel is straight and engages correctly in the threads of the connector.
- 4 Remove the protective cap from the Profibus connector of the Master unit.
- 5 Connect the Profibus cable to the Profibus connector of the Master unit.

#### Power on and set up the system

- 1 Power on the ÄKTAprocess unit and allow 5 to 10 seconds for the boot process to finish.
- 2 Power on the Master unit.
- 3 Log in to UNICORN.
- 4 In system control in UNICORN, disconnect ÄKTAprocess.
- In *administration:system setup* in UNICORN, select the component *AxiChrom* **300-1000** for the ÄKTAprocess system.
- 6 In system control in UNICORN, connect the ÄKTAprocess system.
- 7 On the screen of AxiChrom Master, select the appropriate column to be used and confirm the bed height.
- 8 Exit to the **MAIN MENU** in the AxiChrom Master unit. The system is now ready to use.

# Disconnecting ÄKTAprocess from AxiChrom Master

- 1 In system control in UNICORN, disconnect ÄKTAprocess.
- 2 Power down the ÄKTAprocess unit.

3



#### **CAUTION**

Make sure that the ÄKTAprocess unit is powered off before the following step is performed.

Power down the AxiChrom Master unit with the *Power Off* button in the *MAIN MENU*.

- 4 Disconnect the Profibus cable from the ÄKTAprocess unit.
- 5 Place the Profibus termination plug on the connector of the ÄKTAprocess unit.
- Remove the Profibus cable from the connector of AxiChrom Master unit and replace the protective cap.
- 7 Power on the ÄKTAprocess unit and log in.

# 3.7 Using the Master

## 3.7.1 Start Master and calibrate column

# **Description**

Every time the AxiChrom Master is started, the column list is shown. When a column has been chosen, the *Calibration* page is displayed and the driver will automatically be calibrated to the column motor. After calibration the *Verify adapter position* page is displayed. Enter the adapter position as read on the column scale.

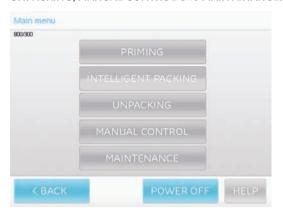
#### Firmware installation

AxiChrom Master comes fully installed with the firmware needed. To support new GE Healthcare media for Intelligent Packing or improved functionality (including new column models), AxiChrom columns ordered as separate units after AxiChrom Master delivery may have later firmware versions enclosed including compatibility information and installation instructions. Please contact your GE Healthcare representative for further information.

# 3.7.2 User interface

## Master wizards

The *MAIN MENU* is the starting point for wizards for *PRIMING*, *INTELLIGENT PACKING*, *UNPACKING*, *MANUAL CONTROL* and *MAINTENANCE*.



# **Buttons and explanations**

Button	General interface functions
<b>MAIN MENU</b> or <b>MENU</b>	Closes the ongoing operation and opens the <b>MAIN MENU</b> dialog. The button is active when it is grey.
	PRIMING—guides the user through safe priming of the column.
	INTELLIGENT PACKING—with pre-programmed packing methods it guides the user through packing of the column to achieve optimal compression and bed efficiency.
	UNPACKING—guides the user through safe unpacking of media from the column.
	MANUAL CONTROL—enables the user to control the adapter movement and open or close the Media valve.
	MAINTENANCE—guides the user to set the column into Maintenance mode, from where safe service of the column can be performed. See AxiChrom Operating Instructions.
	POWER OFF—from this fail-safe mode it is safe to turn off Axi- Chrom Master without loss of information.

Button	General interface functions
HELP	Opens contextual help texts/information for the current dialog. From the <i>HELP</i> dialogs it is possible to open a Flow converter, for conversion of linear to volumetric flow. The <i>HELP</i> button is normally grey when it is active. In the column list the <i>HELP</i> button turns blue if the marked column has corrupt column information.
BACK	Opens the previous dialog. The <b>BACK</b> button is blue when it is active.
CONTINUE	Opens the next dialog in the workflow. This button is only active (blue) when all mandatory information has been entered in the active dialog. In some wizards it is possible to press the blue active <i>CONTINUE</i> button before the time bars have been filled. This is to make it possible to speed up the specific process step, but if nothing is done the procedure will continue as normal.
CONFIRM	Opens the next dialog in the workflow, after the stated prerequisites are fulfilled. This button is active (blue) only when all mandatory information has been entered in the active dialog.
SAVE	Saves the entered information and continues to the next dialog. This button is active (blue) only when all mandatory information has been entered in the active dialog.
START	Starts a process (the adapter may move). This button is active (blue) only when all mandatory information has been entered in the active dialog.
Stop	Stops the process manually.
End Filling	Ends the filling step manually and continues with the consolidation phase. Active during the filling when it is grey. If <i>End Filling</i> is used entered parameters might not be met and the packing might fail.
END PACKING	Stops the adapter during the final compression of the bed. <i>END PACKING</i> should be pressed when optimal Packing Factor or desired bed height within given range have been met. This button is green when active.
PAUSE	Stops the process temporarily. Subsequent options are either to abort the process permanently or to continue. Active when it is blue.
ABORT	Aborts the current process and opens <b>MAIN MENU</b> . Active when it is light grey.
Virtual keypad	The virtual keypad is used to enter input for text and figure fields.

# Information in progress dialogs

During packing, unpacking and priming, the progress can be monitored in progress dialogs. They display:

- · the remaining time and a graphical progress bar
- the adapter position, shown both graphically in the column drawing and in the adapter position box
- actual pressure in the column
- the state of the bottom Media valve
- the packing process steps, shown in text and checked when completed.
- new status messages.

The action buttons will change functions depending on the specific status of the packing process. The status message may call for some action from the user, for example to open the Bottom mobile phase valve or clean the valve and tube. The action will then require a confirmation before the process is resumed.

# 3.7.3 Column and Master setup

#### Select column



- If the column to be used can be seen in the list, select it with the arrow buttons.
- If the column to be used can not be seen in the list, press *Add* to add a column to the list. No column will be listed when Master is delivered.
- Press *Edit* to change the parameters of the chosen column or the column name.
- Press **Delete** to remove the chosen column from the list.

- Press **CONTINUE** when a column is chosen in the list.
- Press **CONFIGURATION** to enter the Configuration wizard from where it is possible to view system settings, system specifications and test the system.

# If the settings are corrupted

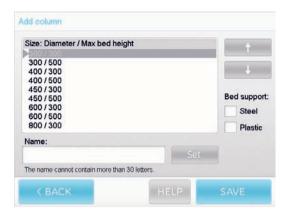
If the *HELP* button turns blue when a column is chosen <sup>1</sup> in the list, the settings for the column have been corrupted. It is not possible to continue using the settings for this column when they are corrupted. This situation can occur when the AxiChrom Master system was last shut down and was not allowed to save the data completely before the Power switch was turned off. If this happens, follow the instructions below to recreate the column settings. Press the *HELP* button for more information.

#### **Recreating column settings**

- 1 Press **Delete** to remove the corrupted column settings.
- Press Add and create new column settings.
- 3 Choose the column parameters for the deleted column in the list. Type a name and press CONTINUE.
- 4 Verify the column parameters according to the wizard instructions.
- 5 Calibrate the motor according to the instructions in the Master.
- 6 Verify the adapter position according to the wizard instructions.

<sup>1</sup> The button also turns blue when the selected line is empty.

#### Add or edit a column



The dialog is reached after pressing *Add* in the previous dialog. Settings for a column can be created here.

- The column diameter and maximum bed height can be found on the column label. It is located on the lower front part of the frame on the column.
- Select the correct column diameter and maximum bed height in the dialog by using the arrow buttons. The chosen column will be highlighted.
- Select the type of bed support fitted in the column to be used. The options are Steel
  and Plastic.
- Press Set to open the on-screen keyboard where a unique column name can be written. This name can contain a maximum of 30 characters.

**Note:** It is recommended that a label with the column name (that was given by the operator in the AxiChrom Master), is placed on the column. The Serial number is a good unique name.

- Press **SAVE** to save the chosen settings for the column and to continue.
- Press **BACK** to return to the Select column dialog without saving. Editing the column name is possible even when the column has been saved.

#### **Motor calibration**



The *Calibration* page will synchronize the Master unit with the motor. The column may not function with the Master unit if the motor is not calibrated. A calibration is therefore always performed when a new column is selected.

Calibration will start automatically and will normally take less than one minute to complete.

When the calibration is completed the **CONTINUE** and **BACK** buttons will turn blue.

- Press CONTINUE to proceed to the position verification page.
- Press **BACK** to return to the column selection page

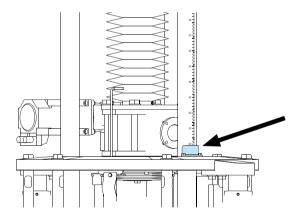
**Note:** If several columns are to be calibrated in a row, the Master unit must be shut down and restarted between calibrations.

# Verify the adapter position

The actual adapter position on the column has to be entered in the AxiChrom Master. Follow the procedure below to correctly read and enter the correct adapter position in the dialog.



1 Verify the actual distance read from the level scale on the adapter rod. Observe that the scale has increasing figures running downwards.



#### Note:

The level scale indicator differs between columns with different bed support material (plastic or stainless steel). Make sure that the correct level scale indicator is fitted.

- Press Set to change the dialog value Last known adapter position if it differs from the actual adapter position, read from the level scale. Set the value to a tolerance of one decimal in centimeters.
- 3 Press **CONTINUE** to enter the **MAIN MENU**.

# Verify the factory calibration value for the level display

- 1 Connect the column and the Master.
- 2 Start the Master.
- Verify that the adaptor value in the master correspond to the value on the Level display label.
- 4 Check the factory Master calibration value (cm) in the functional test records in the column product documentation.
- Move the adaptor upwards using a low speed (30 cm/h) to the end of the stroke. Use the current limitation to stop the adaptor.
- 6 Verify that the adaptor height on the level display scale corresponds to the test record (Height difference < 0.2cm).

# **Master configuration**

#### Description

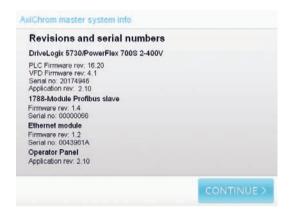
To access the configuration wizards, press the **Configuration** button on the **Select column** page.



Use the configuration wizards to

- run startup tests and access the Panel view (only accessible by a GE Healthcare service operator)
- view system settings (only accessible by a GE Healthcare service operator)
- view system information and specifications

#### **System Info**



This dialog displays serial numbers and firmware revisions. Press **CONTINUE** to proceed.

**Note:** The above image is an example. Other firmware and application revisions may apply.

# **Necessary adjustments**

If	Then
The column is new	Adjust the tightening torque of the bolts on the tie rods, see <i>Tightening torques</i> , on page 87
The column has been moved from a room with a different temper- ature	Adjust the tightening torque of the bolts on the tie rods, see <i>Tightening torques</i> , on page 87
The room temperature has changed significantly	
The column tube or other major parts have been replaced	Contact GE Healthcare to have a service technician perform an adapter position calibration

#### Leakage test

Perform a leakage test as described in the AxiChrom 300-1000 Operating Instructions when:

- the column is new
- the column has been shipped
- the o-rings have been replaced
- other parts that may cause leakage have been replaced
- maintenance has been performed

# 3.8 Cleaning the column

The cleaning should be performed before the bed is packed or when the column is new (that is, when the column is empty or filled with transportation/storage solution).

# Cleaning procedure

Note:

Parts made from non-plastic materials may be autoclaved. For more information about cleaning methods, see the User Manual.

Clean the outside of the acrylic tube with for example water and/or 20% (v/v) ethanol to remove any surface residues from the packaging.



#### NOTICE

Do not use ethanol stronger than 20% (v/v) for cleaning the acrylic tube, since this may damage the column tube.

- 2 Steel surfaces can be cleaned with a wetted cloth and 70% (v/v) isopropanol or 70% (v/v) ethanol.
- 3 Ensure that the adapter seals and o-rings are clean from particles and waste, since these may damage the adapter and tube when the adapter is moving.
- 4 Perform a priming according to the instructions in the **PRIMING** wizard, in the AxiChrom Master **MAIN MENU**. This procedure will clean the column and its tubes and remove any air from the column.
- Ensure that the cleaning solution is completely flushed out and replaced by an appropriate storage solution after priming the column.
  Result: The column is now primed and cleaned.

## Cleaning the Master

Turn off the AxiChrom Master before it is cleaned with water and/or 20% (v/v) ethanol.

# 3.9 Ambient temperature changes

# **Effects of temperature changes**

If the temperature surrounding the column is changed significantly, the acrylic tube will shrink or expand. This will cause unwanted tension in the acrylic tube, in the dome nuts on the adapter, the bolts of the tie rods, and the bottom bed support nuts—i.e. they will become tightened or loosened.

The tightening torque of the adapter dome nuts, tie rod bolts and bottom bed support nuts must be checked whenever the temperature has changed, see *Tightening torques*, on page 87. The bolts should be tightened crosswise in small intervals. Make sure that the torque wrench is calibrated and that the correct socket is used.

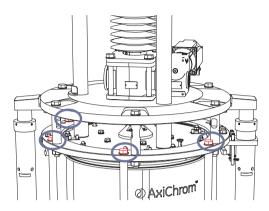
# **General guidelines**

- The column should not be placed in direct sunlight, since this could affect the column temperature.
- The column should preferably be packed and run in a room without significant temperature changes.
- Check the tightening torques of the adapter dome nuts, tie rod bolts and bottom bed support nuts when the column is new and has been placed in its first location.

If the column is moved	Then
<b>From cold to warm</b> surroundings	The adapter dome nuts, the tie rod bolts and the bottom bed support nuts should first be loosened to finger-tight in the colder room and then moved to the warmer room. When the column is properly tempered, after about 24 hours, the adapter dome nuts, the tie rod bolts and the bottom bed support nuts should be tightened according to the tightening torques below.
From warm to cold sur- roundings	Ensure that the column is properly tempered, after about 24 hours. Then tighten the adapter dome nuts, the tie rod bolts and the bottom bed support nuts according to the tightening torques below.

# **Tightening torques**

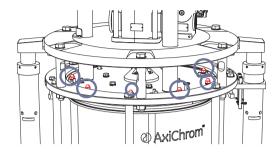
# Tie rod bolts



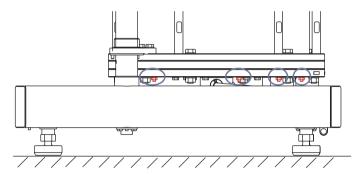
# Tightening torques for tie rod bolts

Column	Tightening torque
300, 400 and 450	20 Nm
600	28 Nm
800	42 Nm
1000	66 Nm

# Adapter dome nuts and bottom bed support nuts Location of dome nuts



# Location of bottom bed support nuts



# Tightening torques for adapter dome nuts and bottom bed support nuts

Column	Tightening torque
300-600	10 Nm
800-1000	15 Nm

# 4 Preparations before packing the column

# 4.1 Packing Factor and Compression Factor

#### Introduction

During a standard AxiChrom packing procedure the media is first settled under a low flow, induced by the adapter moving downwards. This is what we refer to as consolidation. The Top mobile phase is closed and the Bottom mobile phase is open during packing, and the excess liquid is expelled through the Bottom bed support on which the bed will start to form. When all media has settled and formed a bed at the bottom of the column, the bed is consolidated (still under influence of a low flow) until the adapter hits the bed. Adapter movement then continues, compressing the media until the required packed bed height is reached based on the packing factor used. The Packing Factor (PF) is calculated as follows.

packing factor PF = (consolidated bed height) / (packed bed height)

The Packing Factor takes the settling speed of media into account. The Compression Factor (CF) is defined as the ratio when no flow through the bed is applied.

**compression factor CF** = (gravity settled bed height) / (packed bed height)

There is a difference between Packing Factor and Compression Factor. The definition of Packing Factor involves flow through the bed which in some cases leads to a more compressed bed and lower consolidated bed height, while in other cases the gravity settled bed height is lower since the bed packs more densely if given more time to do so. Mabselect SuRe™, for example, has a significantly lower gravity settled height than its consolidated height and this has to be considered when calculating the amount of media to be packed in the column.

# About default packing values

The default values in the Master software are tested and verified parameters for packing in water/20% ethanol. Packing Factor is allowed to vary around the optimal value (±0.03), which has also been verified.

 For HIC media the default values are defined for packing in 10% to 20% ethanol in the AxiChrom 300 to 1000 columns.

#### 4.1 Packing Factor and Compression Factor

- For some media packed in salt-containing solution a higher Packing Factor has to be used. This is due to different settling conditions in solutions other than water and ethanol.
- Note that all S and SP ligand media are shipped and stored in 0.2 M sodium acetate and any decanted solution will contain salt.

For detailed information about which Packing Factor to be used depending on packing buffer, see *Appendix B Packing buffer information*, on page 183.

# **Bed support porosity**

The bed support porosity should be less than or equal to 1/3 of the average particle size of the media. This ensures that all particles are larger than the holes in the bed support. Using a bed support with a larger porosity results in particles passing or getting caught in the screen, leading to poor bed efficiency and, for a clogged screen, high pressure.

# 4.2 Preparation of the slurry

**Note:** Prepare the slurry at least 2 hours before usage in order for it to properly degas after shaking. The slurry can be prepared up to 1 day ahead.

**Tip:** In the case of packing short beds, in the range of 10 to 15 cm, it is often suitable to first reduce the slurry concentration to about 30% to 35%. Further it is recommended to increase the Packing Factor from the set value in the Master by 0.02 or 0.03 (2% to 3%). Using either or both of these modifications often leads to improved results.

#### **Procedure**

- Calculate the amount of media using the formula media volume = target bed height × PF × column cross-section.
- Decant the supernatant and replace with recommended packing buffer according to the tables on page 183 until a suitable slurry concentration is reached.
  - Short column tube: at least 30% for 100 mm bed height and 65% for 300 mm bed height
  - Long column tube: at least 30% for 100 mm bed height and 74% for 500 mm bed height

#### Note:

Minimum concentration = (bed height × PF or CF) / max fill height

3 Determine the slurry concentration, see Section 4.3 Determining the slurry concentration, on page 91.

- Prepare a sufficient amount of slurry in the slurry tank to be able to pack the desired bed height. The required amount of slurry is also automatically calculated in the AxiChrom Master Intelligent Packing wizard and displayed in the summary page before the packing is started. Use CF for gravity settled media.

  Slurry volume = (target bed height × PF × column cross-section) / slurry concen-
  - Siurry volume = (target bed height × PF × column cross-section) / siurry concentration
- 5 Prime the media valve.
- Make sure that all valves in the slurry tank are closed and that the gel can not flow into any tubing (where it will sediment). Use a media stirrer to make homogenous slurry in the tank before packing.

#### **Tip:** For Manual operation:

- Max fill height for short column tubes (bed height 100 to 300):
   570 mm
- Max fill height for long column tubes (bed height 100 to 500): 830 mm

# 4.3 Determining the slurry concentration

#### Choice of methods

Two methods are presented here for determining the slurry concentration:

- Forced settling
- Gravity settling

# Forced settling

To achieve the most accurate result, a general determination method for slurry concentration has been developed. The method utilizes a laboratory column, preferably XK16 or HiScale™ 16, and a small pump that can deliver 100 cm/h for the specific column.

#### **Preparations**

- 1 Mount an end piece/adapter with a filter to the bottom of the column.
- Carefully place a transparent ruler on the side of a clean and empty labscale column so that the zero mark on the ruler coincides with the surface of the bottom filter
- Make sure that there is a stopper at the bottom outlet of the column.
- 4 Secure the column in an upright position.

#### Sampling

- 5 Prepare the slurry in a tank of appropriate size.
- Make sure the gel slurry is homogenous. The best way to achieve this is by stirring. Stir from all directions, with varying tilt. Allow at least 5 minutes for stirring. This is the largest source of error in the method.
- 7 Take a sample from the middle (depth and width) of the batch, preferably using a Falcon 25 ml pipette.

#### Column filling

- 8 If the sample has an appreciably high concentration (>60%), dilute it by first adding 2 cm of water to the column. The accuracy of the method is higher for lower concentrations.
- 9 Add thoroughly mixed gel slurry to the column until the right volume (about the height of 100 mm) is obtained. Use a Falcon (or Pasteur) pipette (25 ml) to avoid resin on the column wall above the 100 mm marking.
- 10 Add water until the glass body is filled.
- 11 Secure the end piece/adapter and the filter on the top of the column.

#### Washing and settling steps

- 12 Connect the column to a pump that can deliver a flow of 100 cm/h for the specific column used.
- 13 Wash the media up-flow with pure water for 5 column volumes.
- 14 Change flow-direction from up-flow to down-flow.
- Let the media consolidate in 1 column volume of pure water, at 100 cm/h.

#### Determining the slurry concentration

- Let the gel bed stabilize for 30 minutes without any flow and then read the bed height.
- 17 Convert the bed height to slurry concentration: slurry concentration(%)=bed height/slurry height\*100 (e.g. a bed height of 58 mm obtained from slurry filled to 100 mm gives a slurry concentration of 58%. Remember to adjust for dilution (step 8) if required (e.g. if 20 mm water is added to the column first, and slurry is added to a final level of 100 mm, divide the calculated slurry concentration by 0.8).

## **Gravity settling**

Gravity settling in a graduated cylinder is one of the most commonly used methods. It is best to use fairly large cylinders for process scale columns, at least 1 liter of slurry.

- Stir the sample so that all media is suspended and the slurry is completely homogenous.
- 2 Quickly remove a sample using a container, for example a beaker.
- 3 Pour it immediately into the cylinder.
- 4 Place it on a flat surface and cover the opening with for example Parafilm, to minimize evaporation.
- 5 Allow to stand at least overnight.
- 6 Read the total volume and the media volume and calculate the slurry concentration (media volume/total volume).
  - Carefully tilt the cylinder and check the media surface.
    - If the surface does not sway, the media is settled well enough.
    - If the surface is not sufficiently settled, it is recommended to allow the sample to stand longer. This occurs for some media, especially small particles such as Sepharose High Performance but also for media that is settled in salt-containing solutions.

# 4.4 Priming the column

#### In this section

This section first gives an overview of the column priming procedure. Later in this section there is a more detailed, stepwise description of how to create and use a UNICORN method or Master to prime the column. The procedure is described for columns with acrylic column tubes, with deviations for stainless steel column tubes where appropriate.

# 4.4.1 Short description of the priming procedure

During the priming procedure the adapter will go to priming position at the top of the column. The column is then filled with priming liquid. When the priming liquid comes out from the column top, the Seal flush valves (see page 98) are opened and the pressure in the column is increased with the connected membrane valve. When the pressure has been increased the adapter will move downwards past the priming groove, expelling air from inside the column through the rinse channel out through the Seal flush valves.

When the adapter stops, the Seal flush valves are closed and the membrane valve fully opened. The adapter then starts to move and the top bed support is primed. When the adapter passes 30 cm from the bottom bed support the priming direction changes and the bottom bed support is primed. When the adapter comes to the bottom of the column and stops, the priming procedure is finished.

Priming is performed either using UNICORN on an ÄKTAprocess system or from the Axi-Chrom Master directly. The workflows are described in Section 4.4.4 Using UNICORN method, on page 97 and Section 4.4.5 Using Master wizard, on page 99.

# 4.4.2 Wetting plastic bed supports

Follow the instructions below if the column is fitted with plastic bed supports.

Note:	Before priming the column, mount the bed supports dry and assemble the
	column as described in the AxiChrom 300-1000 Operating Instructions. Connect
	membrane valves on the column top and bottom mobile phases.

	memorane valves on the column top and bottom mobile phases.
Step	Action
1	Prime the column according to the AxiChrom Master priming instructions or a UNICORN priming method.
2	Perform a leakage test.
3	With the adapter at 1 cm from the bottom bed support, equilibrate the column with 20% v/v 1-propanol at 30 cm/h under a pressure of 1 bar for five column volumes.
4	Pause the flow. Use the membrane valves to seal the column mobile phases and incubate for 2 hours at 1 bar.
5	Carefully release the pressure on the membrane valves.
6	Flush the column with at least five column volumes of deionized water or packing buffer.
7	Pack the column according to the AxiChrom Master or a UNICORN packing method.

# 4.4.3 Creating a UNICORN method

The Method Wizard in UNICORN simplifies creation of a Priming method. The methods require an initial selection of a column dimension. Thereafter the user makes appropriate selections to complete the creation of the method. This section describes the creation of a UNICORN Priming method.

#### Procedure

Start the Method Wizard in the Method Editor window and choose the system in the pop up window.

## 2 Select **Priming**.



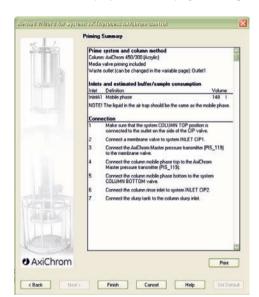
Setting	Description
Column	Select <b>AxiChrom xxx/yyy</b> (xxx = column diameter in millimeter, yyy = max bed height for short and long column tube, 300 or 500 mm respectively). The column size can be found on a label located on the lower front part of the frame of the column tube.
Column material	Select the material of the column tube.

## 4 Preparations before packing the column

- 4.4 Priming the column
- 4.4.3 Creating a UNICORN method
  - 3 Click Next. The wizard page Priming Parameters opens. Select the appropriate inlet.



Setting	Description
Prime media valve and slurry hose	Leave checked to prime the media valve during the priming process.
Inlet for mobile phase	Select the appropriate inlet.



4 Click **Next** to display the wizard page **Priming Summary**.

This page provides a summary of the options selected in the previous wizard pages. The summary page also gives information on buffer volumes and instruction for how to connect the column to the ÄKTAprocess system.

- 5 Click *Finish*. The column packing summary can then be viewed under *View/Run Setup/Notes/Method Notes* in the Method Editor module of UNICORN.
- 6 Choose an appropriate result file folder, result name and save the method.

# 4.4.4 Using UNICORN method

The Media valve, column and system are primed using UNICORN and ÄKTAprocess.

The priming sequence using a UNICORN method is the same as when priming using the Master wizard from Step 5. The difference is that during UNICORN control, manual interactions are minimized to control the Seal flush valves and to perform some confirmations. The ÄKTAprocess system controls other valves, flows and rinsing procedures.

#### Preparations before running the method

Connect the column using the automatic configuration for ÄktaProcess as described in Section 3.6 Column and system setup, on page 68.



#### WARNING

Ensure that nobody is present within the safety zone when the adapter is moving. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.

#### Running the method

- 1 Start the priming method.
  - Result: The system performs a wash procedure. The Media valve and the slurry tubing/tank are then primed (if selected in the Method Wizard dialog).
- 2 Click **Continue** when prompted to do so when the stated conditions have been fulfilled.

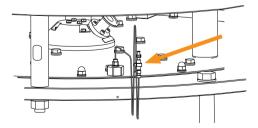
*Result*: When the Media valve has been primed the column is filled with priming solution and the adapter moves to the priming position. If the adapter does not move it is already in the correct position.

#### Note:

With stainless steel column tubes, it is not possible to see when liquid has passed the priming groove. Verify instead that the adapter is in the top position and that buffer flows out of the top mobile phase outlet before clicking **Continue**.

When prompted to do so, increase the pressure in the column by tightening the membrane valve to approximately 0.3 bar, open the Seal flush valves and then click *Continue*. (This will happen when the adapter is in priming position above the priming groove, the column has been filled and priming solution comes out in waste through the system and through the Seal flush valves, and the flow is lowered.)

Result: The adapter then moves down at 40 cm/h to a position under the priming groove expelling the air below the scraper seals as the adapter passes the groove.



4 During the priming, direct the Seal flush valve to waste or to a vessel.

- The adapter stops below the priming groove. When prompted to do so, open the membrane valve slowly to release pressure in the column, close the Seal flush valves, and click *Continue*.
  - Result: The adapter automatically moves down to 30 cm at a velocity of 300 cm/h expelling air and priming solution through the top bed support and the Top mobile phase. At 30 cm the flow direction in the system is automatically changed so that the bottom bed support and bottom mobile phase are primed while the adapter moves down to the bottom position of 1.0 cm.
- The priming of the Media valve, column and system is now complete.
- 7 Exchange the liquid in between the scraper seals to 20% ethanol.

# 4.4.5 Using Master wizard

The Priming procedure described in the wizard guides the operator through a number of steps to prime the column with a standalone pump. Follow the instructions in the **PRIMING** wizard, found in the **MAIN MENU** in AxiChrom Master.

For further explanation of the buttons used in the wizard, refer to *Buttons and explanations*, on page 76. For tubing connections, see *Manual configuration*, on page 69.

**Note:** Before the column is primed, the column must also be calibrated (page 75) and cleaned (page 85), also when priming with UNICORN.



#### WARNING

Ensure that nobody is present within the safety zone when the adapter is moving. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.



#### **CAUTION**

Always open and close external valves slowly. Sudden changes of pressure in combination with air in the column might cause damage to the column

For further explanations of the progress dialogs in the wizards, see *Information in progress dialogs*, on page 78.

#### Step 1: prime the media valve manually

If the column is primed with the guidance from the Master and a standalone pump, the Media valve has to be primed manually. Follow the procedure below to prime the Media valve manually.

#### 4 Preparations before packing the column

- 4.4 Priming the column
- 4.4.5 Using Master wizard
  - 1 Connect a pump to the Rinse inlet.
  - 2 Connect the slurry tubing/tank to the Slurry inlet.
  - 3 Open the slurry tubing/tank.
  - 4 Close the Bottom mobile phase and the Media valve.
  - 5 Start a flow through the Rinse inlet and further into the slurry tubing/tank, at approximately
    - 200 l/h for 300, 400, 450 and 600 columns
    - 800 l/h for 800 and 1000 columns.
  - When the tubing is free of air: make sure the Top mobile phase is open, and then open and close the Media valve from **MANUAL CONTROL** in the Master a couple of times. To make sure no more air comes from the Media valve, continue to pump liquid through the valve, filling the bottom of the column and covering the bottom bed support with liquid (for example 1 cm). (If the column is packed, avoid opening the Media valve during the valve priming.)
  - 7 When no more air comes up from the Media valve, close it.
  - 8 Stop the pump and close the slurry tank.
  - 9 The Media valve has now been primed.
  - 10 If applicable, calculate the new slurry concentration by adding the amount of packing buffer pumped into the slurry tank to the total volume in the tank.

Note:

To minimize the dilution of the slurry in the slurry container, prime the Media valve and slurry tubing with packing buffer before the slurry container is connected. Then prime the connection with a minimal amount of packing buffer but enough to get rid of any air in the connection.

#### Step 2: select PRIMING from the MAIN MENU

The column is primed when there is no air inside the column and connected tubing. To achieve this, follow the instructions in the *PRIMING* wizard, in the AxiChrom Master. Press *CONTINUE* to start the priming procedure.

**Note:** If the column has not been used for some time add or change the 20% ethanol between the scraper seals.

Preconditions for priming are:

- A pump or a system (e.g. ÄKTAprocess system) is connected to the Bottom mobile phase.
- Both the Top mobile phase and Bottom mobile phase are open.
- The Seal flush valves are open and directed to waste.

- A membrane valve is connected on the outlet of the system used during priming, or
  on the Top mobile phase between the system and the AxiChrom Master pressure
  transmitter PIS 119. The membrane valve shall be open.
- The tubing on the Top mobile phase is directed to waste.

Note:

During the priming procedure, the adapter pushes the liquid out through the mobile phases at up to 300 cm/h. If a system is connected, make sure that it can handle this flow and that the pressure drop in the system is not too high at that flow. The flow deviation alarm and the air alarm in UNICORN (or alarms of equal functionality in other systems) must be disabled, since they may be triggered by a velocity higher than that the system is designed for, or by air bubbles flowing through the system.

Do not disable the pressure alarms on the system! If an alarm is triggered on the system, the adapter must be stopped immediately, otherwise the adapter will continue to move and increase the pressure in the column up to 4 bar, at which the AxiChrom Master pressure alarm goes off.



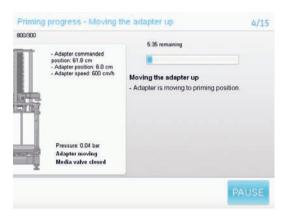
Step 3: enter pump capacity

- Enter the flow that will be used for filling the column by pressing **Set**. The maximum recommended liquid velocity for an unsupported bed support is 400cm/h. Enter the value in liters/hour (I/h), the entered value will then automatically be converted to cm/h.
- 2 Press the **CONTINUE** button to proceed.

#### Step 4: check preconditions

Check precondition boxes when the conditions have been fulfilled. The priming can then be initiated by pressing **START**. The preconditions are the same as when selecting **PRIMING** from the **MAIN MENU** 

Step 5: wait for the adapter to move up

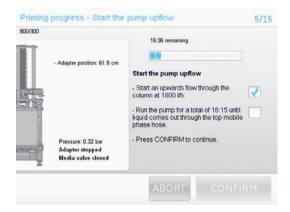


The adapter will now move upward to priming position above the priming groove. If the movement needs to be stopped temporarily, press **PAUSE**.



The priming wizard can also be terminated from here.

#### Step 6: start the pump upflow



- Start an upward flow (i.e. from the bottom to the top) through the column at the stated flow (displayed in the dialog). The time on the time bar will start when the first box has been checked.
- 2 Run the pump for the stated time (displayed in the dialog) or until liquid comes out through the tubing on the Top mobile phase.
- 3 Check the boxes and press **CONFIRM** to continue.

#### Step 7: wait for the buffer to pass the priming groove



- 1 Check the dialog box when the liquid is above the priming groove (found on the inside in the top of the column tube).
- 2 Press CONFIRM to continue.

#### 4 Preparations before packing the column

#### 4.4 Priming the column

#### 4.4.5 Using Master wizard

### Step 8: reduce the flow



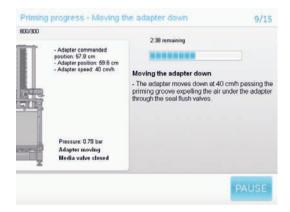
- 1 Set the flow to the value stated in the dialog (100 cm/h).
- 2 Check the box when completed.
- 3 Press CONFIRM to proceed.

#### Step 9: create back pressure



- 1 Create back pressure by tightening the membrane valve on the Top mobile phase until the pressure is approximately the value stated in the dialog.
- 2 Check the box when completed.
- 3 Press CONFIRM to proceed.

Step 10: wait for the adapter to move down past the priming groove



The adapter now moves down at 40 cm/h passing the priming groove, expelling the air under the adapter through the Seal flush valves. Press **PAUSE** to stop the movement temporarily or to terminate the priming wizard.

Step 11: release the pressure



- 1 Close the Seal flush valves.
- 2 Stop the pump.
- 3 Slowly open the valve on the Top mobile phase tubing to release the column pressure. It is important to open the valve slowly when the column is pressurized. When the valve has been opened direct the Top mobile phase to waste.
- Check the boxes and press CONFIRM when all the conditions are fulfilled. The adapter will then start moving at a high speed to prime the top bed support, expelling liquid through the Top mobile phase.

Step 12: wait for the adapter to move down



The adapter now moves down. The remaining time is displayed in a time bar. Press **PAUSE** to stop the movement temporarily or to terminate the priming wizard.

Step 13: prime the bottom mobile phase



- 1 Close the Top mobile phase valve.
- 2 Open the Bottom mobile phase and direct its tubing to waste.
- Press **CONFIRM** when all the conditions have been fulfilled and all the boxes are checked. The adapter will then start moving at a high speed to prime the bottom bed support, expelling liquid through the Bottom mobile phase.

#### Step 14: wait for the adapter to move down



The adapter now moves down to bottom position. The remaining time is displayed in a time bar. Press **PAUSE** to stop the movement temporarily or to terminate the priming wizard

#### Step 15: close the bottom mobile phase valve



- 1 Close the Bottom mobile phase and then check the box in the dialog.
- 2 Press **CONFIRM** to finalize the priming procedure.

#### Step 16: finalize the priming

- 1 The column is now primed. Press **MAIN MENU** to end the priming wizard.
- 2 Exchange the liquid in between the scraper seals to 20% ethanol.

# 5 Packing the column

### In this chapter

This section contains a brief overview of Intelligent Packing and stepwise descriptions of how to create and use a UNICORN method or AxiChrom Master wizard to pack media in the column. Columns are packed according to Intelligent Packing procedures regardless of whether a UNICORN method or Master wizard is used. General instructions deal with packing acrylic column tubes: procedures for stainless steel column tubes are described in Section 5.5.1 Packing stainless steel column tubes, on page 137.

# 5.1 Intelligent Packing

#### Introduction

Intelligent Packing allows for convenient and precise control of the column packing using an AxiChrom column together with an ÄKTA system, UNICORN software, and media with verified packing methods. Several media are available from GE Healthcare that have verified packing methods for use with AxiChrom column and Intelligent Packing. These are found in a list of media in the Method Wizard for Intelligent Packing and in the AxiChrom Master.

For media not included in the Method Wizard, it is possible to create a new packing method in UNICORN (see page 110) or in the AxiChrom Master (see page 135).

# **Intelligent Packing procedure**

Intelligent Packing uses interactive guides to ensure an optimized compression of the bed. Intelligent Packing will pack the column with minimal manual intervention to reproducible results. When the bed has been detected the adapter will proceed to compress the bed until the user is guided to stop the adapter at a specific Packing Factor, which is determined to provide the best results for the selected BioProcess media.



#### NOTICE

In the Intelligent Packing tests, performed by GE Healthcare on its base media selection, the default Packing Factor for each media has been used and verified. The user is therefore recommended to select the default Packing Factor.

For information change the default packing factor, see Section 4.1 Packing Factor and Compression Factor, on page 89.

# Prerequisites for Intelligent Packing

- The column is connected to an Axichrom Master unit
- The column is properly connected to an ÄKTAprocess system or has a manual configuration (see page 68)
- The start-up instructions on page 75 have been followed
- The column is primed (see page 93).
- The slurry concentration has been determined (see page 91).

# Short description of the packing procedure

The packing procedure starts with filling of the column with slurry from the slurry tank. When the correct amount has been filled the adapter starts to go down consolidating the bed on the bottom bed support. When all media have consolidated and the adapter meets the bed surface, the bed contact is detected and the operator shall confirm the detection within 30 seconds. After the detection the compression continues until the desired Packing Factor and/or bed height has been reached. The operator is then prompted to end the packing.

# 5.2 Creating a UNICORN method

### **Background**

The Method Wizard in UNICORN simplifies creation of an Intelligent Packing method. The verified methods require an initial selection of a listed medium. Thereafter the user makes appropriate selections to complete the creation of the method. This section describes the creation of a UNICORN Intelligent Packing method. If a custom medium is selected in the Method Wizard media list, wider range of variables are allowed to be defined to optimize the method, see *Creating a packing method based on custom media*, on page 115. For information concerning low bed heights, see *Section 4.2 Preparation of the slurry*, on page 90.

Note:

Default values for variables in the Method Wizard may be changed to optimize an Intelligent Packing method. Changes using the variables page in the Method Editor module should only be made by advanced users.

#### **Procedure**

1 Start the Method Wizard in the Method Editor window and choose a system in the pop up window.

2 Select whether to create a method for *Column Packing, Packing Test*, or both combined.



Setting	Description
Column	Select <b>AxiChrom <xxx>/<yyy></yyy></xxx></b> (xxx = column diameter in millimeter, yyy = max bed height for short and long column tube, 300 or 500 mm respectively). The column size can be found on a label located on the lower front part of the frame of the column.
Column material	Select the material of the column tube.
Show medium type	Select the appropriate medium type.
Medium	Select the appropriate medium. For custom medium, see 5.2.1 Creating a packing method based on custom medium.
Set Default	Sets the default values used in the Method Wizard.

3 Click **Next** to display the **Packing Parameters 1** Method Wizard page. Select the appropriate variables.



Setting	Description	
Target bed height	Enter a value within the designated range.	
Inlet for mobile phase	Select the appropriate inlet that will be used for equilibration buffer when evaluating the efficiency of the packed bed in the column.	
Inner diameter of slurry hose	Select the appropriate diameter.	
Length of slurry hose	Select the appropriate length.	



4 Click **Next** to display the **Packing Parameters 2** Method Wizard page.

Setting	Description
Min bed height limit	Enter the appropriate height.
Inlet for mobile phase	Enter the appropriate height.
Use default Packing Factor	This box is checked automatically, which means that the medium will be packed using a predetermined Packing Factor. Unless specifically stated in the instructions for a particular medium, it is recommended to use the default Packing Factor. Unchecking this option allows the Packing Factor to be changed.

#### Note:

Over-compression during packing can damage the medium. The default Packing Factor for each medium has been tested and verified by GE Healthcare.

5 Click **Next** to display the **Slurry and Packing Test Parameters** wizard page. Make the appropriate selections.



Setting	Description	
Slurry concentration	Enter the estimated slurry concentration. (The measured concentration is entered later, in the start protocol.)	
Equilibration volume	Enter an equilibration volume (measured in CV) within the designated range.	
Use default liquid velocity during equilibration/pack-ing test	Leave checked to use the suggested default liquid velocity.	
Packing test upflow and Packing test downflow	Select whether to perform packing tests up- flow, downflow or both. Both are selected by default.	
Inlet for sample	Select the appropriate inlet for sample solution.	



6 Click **Next** to display the **Column Packing Summary** Method Wizard page.

This page provides a summary of the options selected in the previous wizard pages, containing for example:

- estimated slurry volume, based on the entered estimated concentration
- estimated consumption of buffer and sample
- instructions on how to connect the AxiChrom column to the ÄKTAprocess system
- 7 Click *Finish*. The column packing summary can then be viewed under *View/Run Setup/Notes/Method Notes* in the Method Editor module of UNICORN.
- 8 Choose an appropriate result file folder, result name and save the method.

# Creating a packing method based on custom media

Intelligent Packing methods may also be developed for custom media using the Method Wizard. The wizard facilitates this by providing an extended range of variables that may be modified to optimize the method. For example, it is possible to:

• Select filling speed in a defined range.

- Pack according to a desired Packing Factor. The custom method utilizes the same sequence for packing as the regular method. The adapter must be stopped in the overlap of defined min/max bed height interval and the Packing Factor range.
- Edit detection limits.
- Select flow conditioning with associated variables. Flow conditioning is always performed down flow, unless the flow direction is changed in the variables page.
- Specify desired adapter velocity during packing within defined range.
- Select sample volume within defined range.

#### **Procedure**

- Start the Method Wizard in the Method Editor window and choose a system in the pop up window.
- Select whether to create a method for Column Packing, Packing Test, or the two combined
- 3 Select the appropriate AxiChrom column.
- Select **All** or **Other** media type, and then select **Custom** under **Medium**.



5 Click **Next** to continue in the Method Wizard.

**Note:** Over-compression during packing can damage the medium. The default Packing Factor for each medium has been tested and verified by GE Healthcare.

# 5.3 Packing using a UNICORN method

### **Prerequisites**

This procedure assumes that slurry preparation and column priming have been performed according to the information on pages 90 and 93. The ÄKTAprocess system controls valves, flows and rinsing procedures.

**Note:** Ensure that the liquid levels in the containers are lower than the system outlet,

to avoid siphoning flow.

**Note:** Ensure that the air trap is filled with mobile phase liquid. This is because the

methods created using the Method Wizard for priming are constructed so that the air trap is inline with the column when equilibration and testing operations are being performed. The packing method is built to bypass the air trap.

**Note:** During the packing procedure it is not possible to pause the process when

During the packing procedure it is not possible to pause the process when the consolidation phase has started. The packing procedure can be ended by pressing End in UNICORN or by using the Emergency stop. If a packing procedure is ended, the procedure will be aborted and the medium has to be un-

packed from the column.



#### WARNING

Ensure that nobody is present within the safety zone when the adapter is moving. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.

### Running the method

1 Run the created packing method.

In the *Variables* page, enter the *Measured* slurry concentration, see the figure below and the information in *Section 4.3 Determining the slurry concentration*, on page 91.



Click *Next*, answer the questions and enter a result name. Ensure that the Master is in *MAIN MENU* before continuina.

- 3 Click **Start** to start the method
- When prompted to, open the slurry tank and then click **Continue**. Keep the media homogeneously suspended throughout the entire fill step.
  - Result: The column is filled automatically with slurry from the slurry tank by raising of the adapter. The filling stops automatically and the Media valve closes when correct amount of slurry is filled into the column. The maximum filling speed is 300 cm/h. After the filling, the system rinses the Media valve from slurry back into the slurry tank.
- When the slurry tube is free from slurry, click **Continue**.
- 6 Close the slurry tank valve and click **Continue**.
  - Result: The bed consolidates as the adapter moves down in the column. A warning dialog is displayed three or six minutes before the calculated consolidated bed is reached, depending on media. The warning dialog should be closed before detection of the bed occurs.

Pay attention as the adapter approaches the consolidated bed surface. When the bed is detected, a confirmation dialog appears.

#### Note:

When the adapter comes into contact with the consolidated bed there will be a lag (default 2 seconds) to sense if the detection has occurred or not. When the detection has occurred the operator confirms that the detection has taken place.

8 If the detection of the consolidated bed surface is correct, click **Continue** and be prepared to end the packing.

Result: The bed compresses and the End Packing figure in the UNICORN flow scheme is colorized



The compression can be followed as the colored boxes light up in brighter red or green color depending on the position of the adapter. When the position of the adapter is within the approved bed height range and Packing Factor range, the *Goto End Pack* button lights up green.



9 When the desired Packing Factor is approaching, double-click the Goto End Pack button.

Result: The manual control-pump command window is opened, with **End-Pack** command highlighted.

#### Note:

**Goto End Pack** can be selected by double-clicking at any time during the compression step.

To stop the adapter when the optimal Packing Factor or desired bed height is reached, click on *Execute* to execute the *End-Pack* command.



*Result*: The adapter stops and the system closes the Bottom mobile phase to prevent the column from draining. If a packing test was chosen in the Method Wizard dialog, it starts automatically.

#### Note:

Ending packing early or late will result in a too low or too high Packing Factor, respectively.

When the packing test is completed, open the Evaluation module of UNICORN and perform an integration of the peak using the packed bed height value.

# 5.4 Packing using the Master Wizard

### **Description**

The Packing procedure described in the Master wizard guides the operator through a number of steps to pack the column with manual valves and a standalone pump. Follow the instructions in the Intelligent Packing wizard, found in the *MAIN MENU* in AxiChrom Master.



#### WARNING

Ensure that nobody is present within the safety zone when the adapter is moving. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.

# Step 1: Select INTELLIGENT PACKING in the MAIN MENU



The AxiChrom Master Intelligent Packing wizard starts with a welcome dialog where all necessary preparations before packing are listed. You can step back and forth in the wizard until the *START* button has been pressed (step 8). The preconditions for packing are:

- The system/pump is primed.
- All tubing leading to the column are primed.
- The column is primed.
- A slurry tank is connected to the Slurry inlet.
- A tube from the pump/system is connected to the Rinse inlet.
- The adapter is in its bottom position.

Press the **CONTINUE** button to proceed.

### Step 2: Select the media type



Select a medium type for a certain chromatography technique using the arrow buttons. The selection All types will list all available media regardless of their applications.

Press the **CONTINUE** button to proceed.

# Step 3: Select the medium



Select an available medium for the selected chromatography technique that was chosen in the previous dialog. A scrollbar will appear on the right side of the list if it is too long to fit on the screen. All lists include a selection called *Other* which is to be used for media other than GE Healthcare BioProcess media.

Press the **CONTINUE** button to proceed.

# Step 4: Enter the hose dimensions



- Enter the slurry tube inner diameter in millimeters, by pressing **Set** if the current value is wrong.
- Enter the slurry tube length in meters, by pressing Set if the current value is wrong.
   Note: If the wrong tubing parameter is set, the bed height can be affected.
   Press the CONTINUE button to proceed.

# Step 5: Enter the target bed height and the slurry concentration



- Press Set if the current value is wrong, and enter the measured slurry concentration in percent. If a slurry concentration above 70% is entered the default filling speed will be decreased to 200 cm/h.
- Enter the target bed height in centimeters, by pressing **Set** if the current value is wrong. The value is set to one decimal place accuracy.
- The deviation from the desired target bed height is automatically suggested by Intelligent Packing bed height limits. The default range is ±1 cm of the target bed height. Press Set to adjust the values.

**Note:** The more accurate the slurry concentration is, the closer the bed height will be to the nominal target bed height. Set a range for the bed height limit to allow some tolerance in slurry concentration.

Press the **CONTINUE** button to proceed.

# Step 6: Confirm the packing settings

Confirm the packing settings. Use the recommended default values for **Packing Factor**, **Filling speed** and **Packing speed**. These have been verified for GE Bioprocess media.



- Press the CONTINUE button to use the default settings that have been verified to give well-packed beds.
- Press *Edit* to change any of the values (not recommended other than for reasons stated in *Section 4.2 Preparation of the slurry, on page 90*). Then, press *Set* in the next dialog to change:
  - Target Packing Factor
  - Filling speed [cm/h]
  - Packing speed [cm/h]

Note:

The recommended filling speed is 300 cm/h for optimal mixing in the column during the filling step. However, if the slurry concentration is high (>70%) or if the slurry viscosity is high (for example in a cold room <10°C), the filling speed should be reduced to 200 cm/h. If the filling speed is too high, cavitation may occur, leading to formation of air bubbles inside the column.

The following settings are used for bed detection and may be changed for packing media **not** in the list on page 183:

- Bed detection delay [s]
- Bed detection level [mbar]

Press the **CONTINUE** button to save the changed settings, or press **BACK** to return without saving.

### Step 7: Verify the settings

Verify the settings. The *Summary* dialog displays all selections made in the previous dialogs and also the calculated required slurry volume in liters.



Press the **CONTINUE** button to confirm the settings, or press **BACK** to return without saving.

# Step 8: Check the preconditions for packing

Check the preconditions for packing.



- The tubing, system, column, and Media valve are primed.
- The Rinse inlet is connected to a pump and is closed.
- Both the Top and Bottom mobile phase valves are closed.
- A slurry tank is connected to the **Slurry** inlet on the column.
- The valve on the slurry tank is opened.
- The media is kept homogeneously suspended throughout the filling process.
- The slurry volume is at least the specified value.

Check the boxes and press START to initiate the packing process.

### Step 9: Fill the column



- The adapter moves upwards.
- Keep the media homogeneously suspended.
- The adapter stops and the Media valve closes automatically when the filling is finished.
- If the pressure falls below -0.30 bar g, the filling will stop until the pressure has risen above -0.25 bar again.
- The adapter speed can be controlled using the arrow buttons. If the adapter stops repeatedly, the slurry concentration is too high and the adapter filling speed needs to be lowered. The speed range limit is 100 to 300 cm/h.
- If the filling for some reason needs to be ended earlier, press the END FILLING button
  to proceed using the pre-defined values—with the risk of ending with a result that
  is out of range for the defined bed height. END FILLING can also be used to abort
  the packing.
- Press PAUSE to temporarily pause the procedure. In the next dialog, press ABORT to quit packing or CONTINUE to proceed.

### Step 10: Rinse the media valve



- 1 Keep the valve on the slurry tank open.
- 2 Start the pump at the stated flow, through the rinse tube and back into the slurry tank.
- 3 When the slurry tube has been rinsed free from slurry:
  - Stop the pump.
  - Close the valve on the slurry tank.
- 4 When all above conditions are fulfilled and their boxes have been checked, press CONFIRM to proceed to the next step.

# Step 11: Open the bottom mobile phase valve



- 1 Ensure that the Top mobile phase valve is kept closed.
- 2 Open the Bottom mobile phase valve and check the box in the dialog.
- 3 Press **CONFIRM** to proceed with packing.

### **Step 12: Consolidation phase**



The consolidation starts. An approximate total time to the low bed height limit and to the calculated bed detection are displayed in the right frame. The current position and the speed of the moving adapter are displayed in the left frame.

#### **Bed detection**

#### Waiting for detection



This dialog will automatically open 3 to 6 minutes before the calculated bed detection occurs. The time depends on the media, with longer time for High Performance media. When the adapter reaches the bed, the user has two options:

- Let the AxiChrom Master detect the contact between the adapter and the gel bed.
- Manually detect, that is, visually verify that the adapter has contacted the bed by pressing *Detect* to proceed to the next step in the packing procedure.

**Note:** Manual detection should only be used if the user is certain that the adapter has indeed come into contact with the consolidated bed, but no automatic detection was executed.

The Axichrom wizard will apply the compression specified for the selected medium starting from the point of execution of the selected option.

#### Bed is detected



There will be a 2 second delay before the bed detection dialog appears. A consolidated bed has been detected automatically or manually.

- Press Confirm to acknowledge the detection and continue to the next step. If not confirmed, the process will stop automatically in 30 seconds.
- Press Ignore if the adapter is not in full contact with the bed. The bed search will
  continue.

#### Unconfirmed detection



The adapter has stopped automatically since the bed detection not was confirmed manually. The actual Packing Factor is displayed.

#### No bed was detected



The adapter has stopped at the low bed height limit without finding any bed. This may happen if the wrong slurry concentration has been set.

Press **Abort** to end the Intelligent Packing wizard and return to the **MAIN MENU**.

### Bed height out of limit



The specifications for bed height range and Packing Factor range can not be met. That is, the bed was detected but the specified bed height range is out of limits from the Packing Factor range, optimal Packing Factor  $\pm 0.03$ .

Press Abort to end the Intelligent Packing wizard and return to the MAIN MENU.

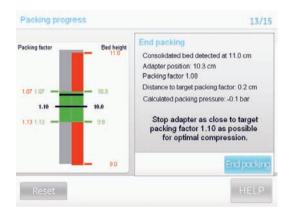
### Step 13: Monitor the compression



The compression of the bed has now started. The accepted Packing Factors are shown on the left side of the animation and the bed height limits are shown on the right side of the animation. The area in which these two criteria overlap, shown as green in the animation, is where an approved packed bed can be achieved. The adapter, shown as the black horizontal line, is moving down in the animation, closing in on the green approved range of Packing Factor and bed height.

**Note:** If the picture for some reason does not resemble this example above, press **Reset** to refresh the picture.

### Step 14: Finish the packing



When the adapter is within the limits (within the green area) the new button *End packing* will appear. Press *End packing* to stop the adapter as close to the target Packing Factor (the static black line in the green area) as possible, for optimal compression.

#### If the adapter is not stopped

If the adapter is not stopped, a dialog like the following is displayed:



The adapter stops automatically

- 15 seconds after the optimal Packing Factor(PF)±0.03 is reached, or
- at the low bed height limit.

In the first case the bed has been over-compressed. Press *Confirm* to proceed. Press *Abort* to end the wizard and return to the *MAIN MENU*. See *Method 2, on page 139* for help about over-compression.

# Step 15: Review the process summary



This dialog summarizes the packing process and displays these values:

- Resulting bed height and target bed height.
- Column volume. The column volume is calculated using several decimal place's
  precision in both the value of Pi and adapter position. This may lead to a discrepancy
  in the displayed volume compared to that which can be calculated from the figures
  shown.
- Resulting Packing Factor and target Packing Factor.
- Height where the consolidated bed was detected.

Press the MAIN MENU button to leave the AxiChrom Master Intelligent Packing wizard.

# 5.5 Creating custom packing methods in the Master

# Methodology for choosing packing parameters

Using the Intelligent Packing wizard, the factors that can be varied to optimize the packing results for a particular media are mainly Packing Factor and packing speed. The packing buffer may sometimes also play a significant role. For more information about packing factors and buffers, see Section 4.1 Packing Factor and Compression Factor, on page 89 and Appendix B Packing buffer information, on page 183.

- Investigate what parameters are suitable for the media. Check literature with the manufacturer, or data from smaller scale columns. It is very likely that the Packing Factor, packing buffer or the compression can be found or at least estimated.
- In the standard Packing wizard (see Section 5.3 Packing using a UNICORN method, on page 117), enter the known Packing Factor or a low value where it is known that there is no damage incurred to the media.
- The packing speed is mainly dictated by the size of the media particles, and potentially also their density and rigidity. In general, smaller and less rigid media in the range from 15 to 40 µm in diameter are suitable to pack at 30 cm/h while larger particles around 100 µm are best packed at 60 cm/h. Very large, rigid and dense particles can be packed at 90 cm/h, even though 60 cm/h often works well.

### **Changing parameter settings**

If the parameter settings for the media are known (Packing Factor, packing speed and filling speed), use the wizard for Intelligent Packing.

#### **Procedure**

- 1 In the *Select media* type dialog: if the separation technique is known, choose the technique, otherwise choose *All types*.
- 2 In the **Select media** dialog, choose **Other**.
- In dialog no. 6/15, press *Edit* to reach the template parameter settings. They are deliberately set rather low for most media. Press Set and enter the parameter settings found for the media.
- 4 Proceed as usual according to the instructions in Section 5.4 Packing using the Master Wizard, on page 120.

### Manual packing

**Note:** This method is recommended for advanced users only.



If the standard method does not fulfill the requirements, the **MANUAL CONTROL** wizard can be used for packing, either as it is or with the help of a standalone pump. All interactions need to be performed manually and without the help of the software interface. Through manual control, the following can be performed:

- Open or close the Media valve (press **OPEN** or **CLOSE**).
- Control adapter movement (press Jog Up or Jog Down).

- Set an adapter position (press Set and then RUN to start the adapter movement.
   Press STOP to stop the movement). The position can be changed again while the adapter moves. If the position is changed during adapter movement the RUN button will be lit up. To verify that the position is to be changed press the RUN button.
- Change the speed (press **Set**). The adapter speed can be varied from 10 to 600 cm/h.

There are two height limits in the manual mode for the adapter. The lower limit is at 1 cm from the bottom. The upper limit is the priming position above the priming groove, which should be used for priming the column. Maximum filling heights are listed in the table below. If the adapter is forced past a limit the adapter will stop automatically. When filling the column manually pay attention not to move the adapter past the priming groove.

	300-600 columns	800-1000 columns
Short column tubes (bed height 100-300 mm)	570 mm	578 mm
Long column tubes (bed height 100-500 mm)	830 mm	838 mm

# 5.5.1 Packing stainless steel column tubes

# Packing the column using a UNICORN method

For the general packing procedure using a UNICORN method, see Section 5.3 Packing using a UNICORN method, on page 117.

With stainless steel column tubes, it is not possible to see when the adapter is approaching the consolidated bed surface (step 7). In order to verify bed detection, observe the pressure on sensor PRESS\_119. Set the vertical scale on the pressure graph for PRESS\_119 to minimum 0.1 bar, maximum 0.5 bar and make sure that the horizontal axis displays time. Automatic bed detection is triggered by a drop in pressure by 0.01 bar for a duration of at least 2 seconds. Manual bed detection is indicated by a corresponding drop in the pressure graph.

#### 5.5.1 Packing stainless steel column tubes

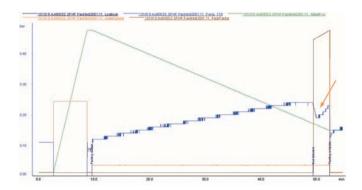


Figure 5.1: The blue trace shows the pressure recorded at PRESS\_119 during column packing. Bed detection is indicated by a transient pressure drop (arrow).

# Packing the column using the Master wizard

For the general packing procedure using the Master wizard, see Section 5.4 Packing using the Master Wizard, on page 120.

With stainless steel column tubes, it is not possible to see when the adapter is approaching the consolidated bed surface (step 12). For manual bed detection, verify that the adapter has reached the consolidated bed by watching the pressure indication in the Master dialog. Automatic bed detection is triggered by a drop in pressure by 0.01 bar for a duration of at least 2 seconds. Manual bed detection is indicated by a corresponding drop in the pressure graph.

# 5.5.2 Post packing modifications

#### Method 1

If there is reason to believe that the bed needs further compression, for example if the asymmetry factor is high or the bed stability is insufficient, the packed bed can be further compressed manually. This is providing that the resulting lower bed height is allowed.

#### **Procedure**

- 1 Enter the **MANUAL CONTROL** wizard from the **MAIN MENU**.
- Determine how much further to compress the bed. Note that over-compressing the bed may cause damage to the particles. As a general recommendation, the bed should not be compressed more than 0.05 higher than the recommended default value in the Master.
- 3 Enter a low adapter speed, 30 cm/h is suggested.

- 4 Open the Bottom mobile phase pathway. The Top mobile phase shall be closed.
- 5 Enter the lower target bed height and press return in the numeric pad.
- Press **RUN** and the adapter will move down to the set height at the set speed, expelling excess liquid through the Bottom mobile phase valve.
- 7 When the adapter stops, close the bottom mobile phase pathway.
- 8 Evaluate the column performance, see Chapter 6 Evaluating column performance, on page 141.

#### Method 2

If the bed was over-compressed it can often be expanded in a controlled way. Results are normally good, but cannot be guaranteed.

#### Procedure

- 1 Start a liquid flow upflow through the column, at least two times higher than the adapter speed in step 2 (that is, >60 cm/h).
- 2 Enter a low adapter speed, 30 cm/h is recommended.
- 3 Enter the target height, higher than the current height but within the earlier applied compression range.
- 4 The adapter will move to the target height.
- When the adapter stops, stop the liquid flow through the column.
- 6 Close the inlet.
- 7 Evaluate the column performance, see Chapter 6 Evaluating column performance, on page 141.

#### Method 3

Another post packing modification is flow conditioning, suitable if

- ullet the bed shows signs of over-compression at one end, such as markedly different HETP and  $A_s$  results in the upflow and downflow directions
- there are cracks in the bed
- HETP test results indicate a bed with insufficient efficiency. Flow conditioning can sometimes correct this
- there are shoulders on the HETP peak, which indicates poor packing

#### 5.5.2 Post packing modifications

For more information about HETP calculation, see *Chapter 6 Evaluating column performance*, on page 141.

To flow condition the bed it is important to have a pump that can supply a flow high enough to create a slight head space of clear liquid above or below the bed. If this cannot be achieved, run the pump, media and column at the highest flow they can withstand.

#### Procedure

- Start the pump in one direction through the column and slowly increase the flow to at least the operating velocity of the process, but preferably more.
- Continue to slowly increase the flow until a small head space is formed in the top or bottom of the column. Make sure that the pressure rating of the column or the media is not surpassed.
- 3 Maintain this velocity for 3 column volumes and then slowly lower the velocity again. Make sure that there is no sudden decrease in the flow, since this may damage the structure of the bed.
- 4 If necessary reverse the flow and repeat the procedure.

# 6 Evaluating column performance

#### Introduction

The efficiency of a column depends on, for example, how well it is packed. A poorly packed column gives rise to uneven flow, resulting in zone broadening and reduced resolution. It is therefore important to have a method by which the column can be tested before it is put into operation. Such a method should be simple, quantitative and should not introduce contaminating materials. It is also an advantage if the same method can be used to monitor column performance over its working life so that it is easy to determine when the medium should be repacked or replaced.

Experience has shown that the best method of expressing the efficiency of a packed bed is in terms of the height equivalent to a theoretical plate (HETP) and the peak asymmetry factor ( $A_s$ ). These values can be determined easily by applying a sample of for example acetone or salt solution to the column.

It is important that the column is properly equilibrated before the packing is evaluated.

# 6.1 Choice of test samples for columns

A solution of either acetone or salt solution, for example NaCl, can be used to give a good indication of the column packing quality. The eluate is monitored by measuring the UV absorption at 280 nm or the conductivity. The resulting elution profile is used to calculate the HETP value and asymmetry.

Both acetone and NaCl may interact with the matrix. Do not use acetone together with the techniques HIC and RPC. If using NaCl on charged media such as ion exchangers, the bed must first be equilibrated with an eluent containing NaCl to a conductivity of about 35-40 mS/cm before applying a higher concentration NaCl sample. The sample is eluted by the equilibration eluent. Consider the instructions for each specific media. Alternatively, the running buffer concentration can be increased 3-fold and used as sample solution, provided that the running buffer contains salt to a concentration high enough that the sample does not interact with the matrix. See Appendix for additional literature.

# 6.2 HETP and asymmetry calculation

### **General guidelines**

The sample volume should be approximately 1 to 2% of the total bed volume, and 1 to 2% v/v acetone can be used. Alternatively 0.8 M sodium chloride, or equivalent can be applied to a 0.4 M NaCl elution buffer, depending on the media that is used. Use a fluid velocity 30 to 40 cm/h for 34  $\mu m$  and 50  $\mu m$  media and 20 cm/h for 75 and 90  $\mu m$  media. Running the HETP test at a higher velocity or using a larger sample volume will affect both HETP and AS values. The airtrap and any filters should be bypassed during the sample application to avoid back-mixing.

An application note describing column testing is available from GE Healthcare.

#### **HETP** calculation

Calculate the height equivalent of a theoretical plate (HETP) and asymmetry factor  $(A_S)$  from the chromatogram as follows:

$$HETP = L/N$$

$$N=5.54 (V_R/W_h)^2$$

Variable	Meaning
L	Bed height
N	Plate number
V <sub>R</sub>	Retention volume
w <sub>h</sub>	Peak width at half peak height

 $V_R$  and  $w_h$  are in the same units

To facilitate comparison of column performance, the concept of reduced plate height (h) is often used. Reduced plate height is calculated as:

$$h = HETP/d_n$$

where  $d_n$  is the mean diameter of the beads.

As a guideline, a value of h<3 is normally well acceptable at the optimal test conditions presented above.

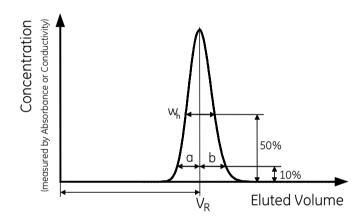
### **Asymmetry calculation**

The peak should be symmetrical and the asymmetry factor as close to 1 as possible (values between 0.8 and 1.8 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration.

The asymmetry factor  $A_s$  describes the deviation from an ideal Gaussian peak shape and is calculated from the peak width at 10% of peak height:

$$A_s = b/a$$

The following test chromatogram shows the parameters used to calculate peak broadening and peak symmetry.



# 7 After the packing

# 7.1 Cleaning-in-place (CIP)

### **Description**

Efficient cleaning methods should be developed as an integral part of the complete process to maximize the working life of the medium. CIP removes precipitated material, strongly bound substances and other contaminants from the medium without the need to disassemble the column. Regular CIP between batches helps assure the desired product quality and the expected working life of the medium and equipment.

For more information about which chemicals to use for CIP, see the table on page 37.

**Note:** Ensure that the media and the column withstands the chemicals used for CIP.

# 7.2 Sanitization-in-place

### **Description**

Sanitization is the use of chemical agents to minimize microbial contamination.

A sanitization procedure is used when there is a need to ensure microbial reduction, for example before changing a product or when there has been unwanted microbial contamination.

Low fluid velocity (60 cm/h) is used for the whole process.

**Note:** Before the sanitization method is started, check that the media withstands 1.0 M NaOH.

# **Regular cleaning**

Regularly wipe the outside of the column using a cloth moist with an antibacterial agent. This reduces the risk of microbial growth.

# General method for sanitization-in-place

- 1 It is recommended to rinse the column and Media valve with purified water, 2 column volumes upflow at a flow velocity of 60 cm/h.
- Clean the Media valve by filling it through the Rinse inlet and out through the Slurry inlet with 1 M NaOH. Let stand for 4 hours before neutralization. Meanwhile, continue with the next step.
- 3 Flush the column upflow with 2 column volumes of 1.0 M NaOH at a velocity of 60 cm/h
- 4 Flush the column downflow with 2 column volumes of 1.0 M NaOH at a velocity of 60 cm/h.
- 5 Recirculate 5 column volumes of 1.0 M NaOH, upflow for 4 hours at a velocity of 60 cm/h
- 6 Wash the column and Media valve with buffer or water of desired quality to achieve a neutral pH.
- 7 Re-equilibrate the column with storage or starting buffer.

An application note on sanitization is available from GE Healthcare.

## 7.3 Unpacking the column

#### **Section contents**

This section first gives an overview of the unpacking procedure. Later in this section there is a more detailed, stepwise description of how to use a UNICORN method or the Master to unpack the media from the column.

#### **Recommended unpacking solution**

The recommended unpacking solution is water. If the media is to be stored, unpack in appropriate storage solution such as 20% ethanol, 0.2 M sodium acetate in 20% ethanol or 10 mM NaOH

## 7.3.1 Short description of the unpacking procedure

There are two unpacking procedures that can be used for unpacking media from the column. If unpacking a Bioprocess media listed in UNICORN or the Master the correct method is chosen automatically. The different procedures are the regular procedure described below used for e.g. MabSelect, Capto and Fast Flow media, and the advanced method for e.g. gel filtration and High Performance media. The difference between the methods is flow direction in the expansion step and number of repetitions of media push out steps. If custom media is to be unpacked see *Creating a custom unpacking method, on page 149*.

The regular unpacking procedure consists of three steps; expansion, collapse and slurry push out. The expansion is performed by lifting the bed by moving the adapter up at the same time as flow comes from the bottom bed support. When the bed has expanded the flow is reversed to collapse the bed by pushing it down. When the collapsed bed has fallen to the bottom of the column the Media valve opens and the slurry can be pushed out from the column by having a low upwards flow into the column and at the same time lower the adapter to the bottom position.

Unpacking is performed either using UNICORN on an ÄKTAprocess system or from the AxiChrom Master directly. The workflows for unpacking acrylic and stainless steel column tubes are described in Section 7.3.3 Unpacking acrylic column tubes, on page 150 and Section 7.3.4 Unpacking stainless steel column tubes, on page 162 respectively.

## 7.3.2 Creating a UNICORN method

The Method Wizard in UNICORN simplifies creation of an Unpacking method. The methods require an initial selection of a listed medium. Thereafter the user makes appropriate selections to complete the creation of the method. This section describes the creation of a UNICORN unpacking method. If a custom medium is selected in the Method Wizard media list, a wider range of variables are allowed to be defined to optimize the method for two different unpacking procedures that can be used, see *Creating a packing method based on custom media, on page 115*).

#### **Procedure**

1 Start the Method Wizard in the Method Editor window and choose a system in the pop up window.

### 2 Select Column Unpacking.

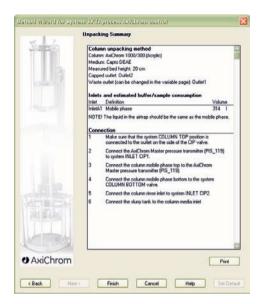


Option	Description
Column	Select <b>AxiChrom <xxx>/<yyy></yyy></xxx></b> (xxx = column diameter in millimeter, yyy = max bed height for short and long column tube, 300 or 500 mm respectively). The column size can be found on a label located on the lower front part of the frame of the column.
Column material	Select the material of the column tube.
Show medium type	Select the appropriate medium type.
Medium	Select the appropriate medium.

3 Click on **Next** to display the **Unpacking Parameters Method** Wizard page. Select the appropriate variables.



Option	Description
Measured bed height	Enter measured value.
Inlet for unpacking liquid	Select the appropriate inlet.
Capped outlet	Select the appropriate outlet blocked with a blind cap.



4 Click on **Next** to display the **Unpacking Summary Method** Wizard page.

This page provides a summary of the options selected in the previous wizard pages. An estimated buffer consumption is given and how to connect the column to the system is described.

- 5 Click on *Finish*. The unpacking summary can be viewed under *Notes/Method Notes* in the Method Editor module of UNICORN.
- 6 Choose an appropriate result file folder, result name and save the method.

#### Creating a custom unpacking method

Unpacking methods may also be developed for custom media using the Method Wizard. The wizard facilitates this by providing an extended range of variables that may be modified to optimize the method.

- Start the Method Wizard in the Method Editor window and choose a system in the pop up window.
- 2 Select the appropriate AxiChrom column.
- 3 Select All or Other media type, and then select Custom medium.
- 4 Click **Next** to continue in the wizard.

For custom media, it is possible to choose from two unpacking modes: regular or advanced.

- Regular unpacking method should be used for media with a medium particle size  $(d_p)$  from approximately 50  $\mu$ m and above. The method consists of a bed collapse part and a slurry push out part.
- Advanced mode should be used for media with d<sub>p</sub> less than 50 µm. The method consists of several bed collapse parts and several slurry push out parts. The sequence for advanced mode is repeated two or three times depending on bed height. A bed height below 25 cm is unpacked with two slurry push outs and a bed height above 25 cm is unpacked with three slurry push outs.

Regular unpacking is the fastest method. If the unpacking behavior is unknown, try the regular method.

## 7.3.3 Unpacking acrylic column tubes

#### Unpacking acrylic column tubes using UNICORN method

This procedure assumes that the column, system and tubing between the system and the column are primed. The unpacking sequence is the same as for the AxiChrom Master in *Unpacking acrylic column tubes using Master wizard, on page 151* from Step 7 "Start the pump upflow". When UNICORN controls the unpacking all valves and flow path are automatically changed. The only operation controlled by the operator is to open the Slurry valve and make some confirmations.

The recommended unpacking solution is water. If the media is to be stored, unpack in appropriate storage solution such as 20% ethanol or 10 mM NaOH.

Note:

One outlet should be capped during the unpacking to block the Top mobile phase outlet during the media push out step when the Media valve is open to the slurry tank.



#### WARNING

Ensure that nobody is present within the safety zone when the adapter is moving. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.

#### Before running the method

Connect the column using the automatic configuration for ÄktaProcess as described in Section 3.6 Column and system setup, on page 68.

#### Running the method

Ensure that the liquid levels in the containers are lower than the system outlet. This is to avoid siphoning flow.

1 Start the Unpacking method.

Result: The bed expands and collapses. To speed up the bed expansion sequence, increase the adapter speed with Pump:Increase\_Adapter\_Speed under Manual Instructions. The adapter speed can be increased in increments of 30 cm/h up to 240 cm/h. Note that the pump flow velocity should always be higher than the adapter speed.

If the bed collapses and comes to the bottom of the column earlier, the command **Next breakpoint** in **Manual/Other** in UNICORN can be used to speed up the process.

- When prompted to do so, open the slurry tank.
  - Result: The System will flow upwards and unpack the media through the Media valve to the slurry tank as the adapter moves downwards. During the last push out step, it is possible to change the adapter speed in
  - **Pump:Increase\_Adapter\_Speed** under **Manual Instructions** to speed up the unpacking procedure. It is not possible to change adapter speed if the pressure is too high, >1 bar or >0.5 bar, depending on media. When the pressure comes below the limit for the media it is possible to change the adapter speed again. The maximum unpacking adapter speed is 240 cm/h.
- When the adapter is at the bottom position a flush of remaining media in the column is initiated. When the bed support is free from media, click **Continue**.

  Result: The Media valve closes and a rinse of the valve and slurry tube is started.
- When the slurry tube is free from slurry, click **Continue**.
- 5 Close the slurry tank valve and click **Continue**.

#### Unpacking acrylic column tubes using Master wizard

The unpacking procedure described in the Master wizard guides the operator through a number of steps to unpack the column with manual valves and a standalone pump. Follow the instructions in the Unpacking wizard, found in the *MAIN MENU* in AxiChrom Master.

For tubing connections see Manual configuration, on page 69.

Note:

This wizard changes depending on the media type and media chosen. The method described below is a general method for the unpacking of Capto and Fast Flow media. A different unpacking method is used for High Performance media. The number of slurry push-out cycles in the High Performance unpacking method depends on the bed height to be unpacked. Two media push out cycles are used for beds lower than 25 cm, and three cycles for beds higher than 25 cm.

**Note:** Always prime the Media valve before operation.



#### **WARNING**

Ensure that nobody is present within the safety zone when the adapter is moving. The operator in charge is responsible for keeping the safety zone free from unauthorized personnel and unnecessary equipment.

#### Step 1: Enter the Master unpacking wizard



- Select UNPACKING in the MAIN MENU to enter the AxiChrom Master UNPACK-ING wizard.
- 2 Ensure that all preconditions are fulfilled before pressing **CONTINUE**:
  - All tubing leading to the column is primed.
  - A pump or a tube from the system is connected to the Rinse inlet.
  - Both mobile phases are opened.
  - A slurry tank is connected to the Slurry inlet.

#### Step 2: Select media type



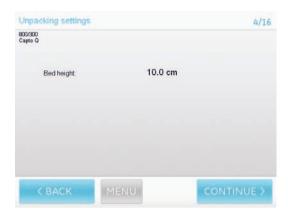
- Select a media type for the chromatography technique that is packed in the column, using the arrow buttons. The selection *All types* will list all available media regardless of their applications.
- 2 Press the **CONTINUE** button to proceed.

#### Step 3: Select media

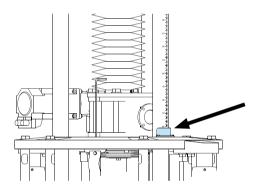


- Select the packed media for the selected chromatography technique that was picked in the previous dialog. A scroll-bar will appear on the right side of the list, if it is too long to fit on the screen. All lists include a selection called Other which is to be used for media other than Bioprocess media from GE Healthcare.
- 2 Press the **CONTINUE** button to proceed.

Step 4: Verify bed height



1 Check that the bed height is correct. The bed height is the same as the adapter position and should always be read from the level scale located on the adapter rod.



#### Note:

The level scale indicator differs between columns with different bed support material (plastic or stainless steel). Make sure that the correct level scale indicator is fitted.

If the value is incorrect, change it by pressing **MENU** and then in the **MAIN MENU**. Press **BACK** to reach the **Verify adapter position** dialog and see Section 3.7.1 Start Master and calibrate column, on page 75 for more information about changing the bed height.

When the values have been confirmed, press **CONTINUE** to proceed to the next step.

Step 5: Connect the pump



Connect a pump to the Bottom mobile phase valve.

Ensure that the pump can be used for both upflow and downflow (connected to either the Top or Bottom mobile phase valves, see *Section 3.6 Column and system setup, on page 68*). It must also provide the velocities in the expansion phase stated in the dialog (listed in the table below).

Table 7.1: Conversion of flow velocities to volumetric flow rates for 30 and 75 cm/h

Column diameter (mm)	Area (cm²)	L/h	
		30 cm/h	75 cm/h
300	707	21	53
400	1257	38	94
450	1590	48	119
600	2827	85	212
800	5027	151	377
1000	7845	236	589

Step 6: Fulfill the conditions before unpacking

Before the unpacking is started, the following preconditions need to be fulfilled.



The required buffer volume is displayed in this dialog. Make sure that this volume is available. Ensure that

- The external system/pump is primed. Ensure that the pump can be used for both up and downflow (connected to either the Top or Bottom mobile phase valves).
- All tubing to the column is primed.
- The flowpath to the column is primed.
- A slurry tank is connected to the Slurry inlet.

Check the boxes in the dialog and press **START** to proceed.

Step 7: Start the pump upflow



- Start the pump upflow through the column at the flow stated in the dialog.
- When the pump has reached a flow of at least the stated value, check the box and press **CONFIRM** to start the unpacking.

#### Step 8: Wait for bed expansion



- Increase the pump flow velocity before increasing the adapter speed. The pump flow velocity should always be higher than the adapter speed. The adapter speed can be increased in increments of 30 cm/h up to 240 cm/h.
- If the vacuum for some reason is below -0.4 bar, the adapter will stop. The flow can be increased in steps of 25 cm/h until the adapter starts to move again. It is then possible to increase the flow to over 75 cm/h until the pressure has reached the target pressure. Use the arrow buttons to control the adapter speed.
- Press PAUSE to temporarily stop the action or to terminate the UNPACKING wizard and return to the MAIN MENU.

Step 9: Reverse the pump flow when the bed is expanded

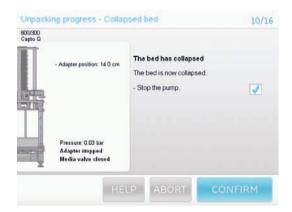


The bed is now expanded.

#### 7.3.3 Unpacking acrylic column tubes

- 1 Reverse the flow through the column and run it at the flow stated in the dialog. This will move the expanded bed towards the bottom of the column. It is possible to continue the unpacking before the time has elapsed if the bed already has collapsed to the bottom of the column.
- 2 Run the pump for the time stated in the dialog.
- 3 Check the boxes and press **CONFIRM** to proceed.

#### Step 10: Stop the pump



- 1 Stop the pump.
- 2 Check the box and press **CONFIRM** to continue to the next dialog.

#### Step 11: Empty the column



1 Open the slurry tank valve.

- 2 Close the Top mobile phase inlet/outlet.
- 3 Start an upwards flow through the column at the flow stated in the dialog.
- 4 When this flow has been reached, check the boxes and press CONFIRM to proceed.

#### Step 12: Wait for unpacking to the slurry tank



- Use the arrow buttons to reduce the adapter speed if the adapter comes too close to the bed. Make sure the distance between the bed and the adapter is at least 2 cm.
   The adapter speed range depends on the media that is unpacked. The largest range is from 30 cm/h to 240 cm/h.
- If the adapter movement stops due to the electric current in the motor rising too high, the method is aborted and an alarm is raised. The unpacking must then be restarted from the beginning or be continued manually.
- If the pressure exceeds the maximum allowed, the adapter movement will temporarily stop until the pressure has dropped.
  - When unpacking bead size  $d_p > 50 \, \mu m$  e.g. Capto, Fast Flow or MabSelect media, the adapter will stop if the pressure reaches 0.5 bar and start to move again when pressure falls below 0.2 bar.
  - When unpacking bead size  $d_p$  <50  $\mu m$  e.g. High Performance media, the adapter will stop if the pressure reaches 1.0 bar and start to move again when pressure falls below 0.5 bar.

The pressure will decrease automatically when the adapter movement has stopped. If the pressure does not drop automatically, ensure that:

- The valve on the slurry tank is open.
- The slurry tube is not clogged.
- The flow is not too high.

#### 7.3.3 Unpacking acrylic column tubes

• If the adapter has stopped due to high pressure it is not possible to change the adapter speed. The speed can be changed again when the adapter starts to move.

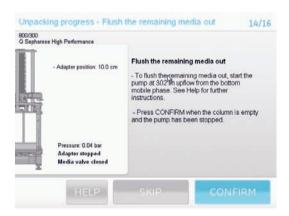
Press **PAUSE** to stop the movement temporarily or to terminate the **UNPACKING** wizard.

#### Step 13: Stop the pump



- Stop the pump when the adapter is in its bottom position. If the pump is not stopped, the slurry will be diluted.
- 2 Press CONFIRM to acknowledge.

#### Step 14: Flush out the remaining media



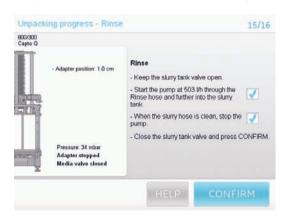
If there is media left in the column when the adapter has come to the bottom position it can be flushed out. Use longer flushing times for more efficient media removal. Collect the flushing liquid in a separate vessel to avoid diluting the previously unpacked slurry.

Press **SKIP** if you do not want to flush out the remaining media.

Follow the steps below to flush out the remaining media.

- Start the pump upflow (from the Bottom mobile phase) using the flow stated in the dialog.
- When the column is empty and the pump has been stopped, press **CONFIRM** to continue to the next step.

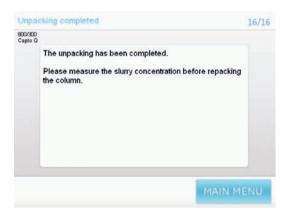




Rinsing the Media valve will clean the valve and slurry tube from media after the unpacking.

- 1 Ensure that the valve on the slurry tank is open.
- 2 Start the pump at the flow stated in the dialog, through the rinse tube, and back into the slurry tank.
- When the slurry tank tube is emptied of gel, stop the pump.
- 4 Close the valve on the slurry tank.
- 5 Check the boxes and press **CONFIRM**.

Step 16: Exit the wizard



- 1 The unpacking is now completed. Measure the slurry concentration before the column is repacked, see Section 4.3 Determining the slurry concentration, on page 91.
- Press MAIN MENU to leave the UNPACKING wizard.

## 7.3.4 Unpacking stainless steel column tubes

#### Unpacking stainless steel column tubes using a UNICORN method

Columns in stainless steel column tubes are unpacked using a UNICORN method in the same way as for acrylic column tubes (see Section 7.3 in the AxiChrom 300-1000 User Manual, edition AB).

#### Unpacking stainless steel column tubes using the Master wizard

For the general unpacking procedure using a UNICORN method, see Section 7.3 in the AxiChrom 300-1000 User Manual, edition AB.

With stainless steel column tubes, it is not possible to see when the column bed expands (step 8-9). The unpacking progress can however be followed from the pressure on sensor PRESS\_119. The pressure drops when adapter starts to move upwards and the bed expands under upflow, then rises again abruptly when the adapter is in the top position and the flow is reversed. There will be a further slower increase in pressure as media settles on the bottom bed support. Go to the next breakpoint in the unpacking method when the pressure increase indicates that medium is accumulating on the bed support.

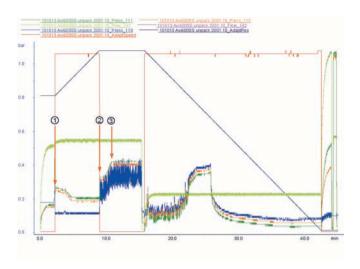


Figure 7.1: 1. The pressure (blue trace) drops as the adapter moves upwards under upflow. 2. The pressure rises again abruptly when the adapter is in the top position and the flow is reversed. 3. The pressure rises further as media settles on the bottom bed support.

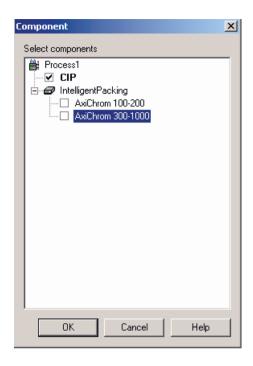
## 7.4 System shutdown procedure

### **Background**

The AxiChrom Master should always be turned off, disconnected and removed after packing, unpacking, maintenance procedures, and before the column is used for separation runs.

#### Note:

If UNICORN has been used to control the Master, make sure that the component in UNICORN System setup/Edit/Component for Intelligent Packing "Axi-Chrom 300-1000" is unticked before disconnecting the Profibus cable as the following image shows.



# Disconnecting ÄKTAprocess from AxiChrom Master

Follow the instructions on page 74.

### Shutdown procedure for AxiChrom Master

Use this procedure to shut off and disconnect the Master safely.



#### WARNING

Disconnect the power cable from the power outlet to AxiChrom Master before disconnecting the power cable between the Master and column. There is still lethal voltage present in AxiChrom Master when it is shut off using the power switch on the Master.



#### WARNING

Ensure that the power cable is disconnected or that the power is shut off at the outlet whenever maintenance work is performed in the Master, to avoid the risk of electrocution.



#### WARNING

A disconnecting system power switch must be installed, such as a branch circuit protection and disconnect switch installed at your facility. The system power switch should be installed at the wall to cut the power to AxiChrom Master. Always use both the Master power switch (located on the back on the Master unit) and the system power switch for safe disconnection of AxiChrom Master.

Note:

When disconnecting the pneumatic pressure leading to AxiChrom Master, a pop can occur. Avoid this by first shutting off the pressure leading to the Master

#### **Procedure**

Press the **POWER OFF** button in the **MAIN MENU**. The dialog **Saving data** with progress bars is displayed briefly as in the following image.



When the following dialog is displayed, use the power switch on the back of the Master to turn off the power.





#### **CAUTION**

If the power switch is turned off before the Saving data dialog has finished, the saved data for the columns may become corrupted and will have to be recreated according to the instructions in Section 3.7.1 Start Master and calibrate column, on page 75.

- 3 Disconnect the Master power cable.
- 4 Disconnect the motor power cable.
- 5 Disconnect the motor feedback cable.
- 6 Disconnect the pneumatic air supply.
- 7 Optionally, dismount the Master pressure transmitter from the flow path.

# Appendix A Parts list and diagrams

Note:

Diagrams showing AxiChrom 600 main column components. The appearance may differ between different column sizes. The table below lists the main parts of the column. See product documentation for full listing.

### A.1 Part numbers and names

Position	Name
104	Tube holder 400 to 1000
105	Tube holder bracket
106	Holder
109	Locking part
111	Fastener, adaptor
115	Fastener, Tube holder 300 to 600
116	Flushing connection
117	Flushing connection nut
126	Plate
127	Shock absorber, lower
128	Top plate
129	Level display
132	Tube Roller, asm
133	Flexible tube 3/4in, TC25
134	Lid support, complete
136	Locking pin, asm
137	Flushing Inlet Tubing L1000
143	Hex Head Screw ISO 4017 M6x25 A4-70
144	Screw M6S-H M5X14

Position	Name
145	Screw M10 X 25
146	Hex Head Screw ISO 4017 M12x45 A4-70
148	Hex Head Screw ISO 4017 M16x40 A4-70
149	Hex Head Screw ISO 4017 M16x45 A4-70
150	Hex Head Screw M12x45
151	Hex Head Screw M12x45
152	Hex Head Screw M12x45
154	Parallel Pin ISO 2338 12m6x60 A2
155	Foot M30
157	Hex Nut ISO 4032 M30 A4-70
159	Domed Cap Nut M10
160	Hex Head Screw ISO 4017 M6x16 A4-70
161	Washer ISO 7089 6.4x12x1.6 A4
163	Washer ISO 7089 13x24x2.5 A4
165	Washer 21x37
169	Washer Nylon 13x24x2.5
170	Washer Nylon 10.5x21x2.5
171	Motor
172	Hex Head Screw ISO 4017 M24x60 A4-70
173	M6S M20x200 A4-70
174	Hex Head Bolt ISO 4014 M16x80 A4-70
175	Hex Socket Head Cap Screw ISO 4762 M10x30 A4-70
178	Hex Socket Head Cap Screw ISO 4762 M8x30 A4-70
181	Hex Nut ISO 4032 M16 A4-70
182	Hex Nut ISO 4032 M8 A4-70
193	Pneumatic Connection Plug
194	Pneumatic Connection Plug

Position	Name
196	O-ring 54x3
197	O-ring 6.02x2.62
198	O-ring 26.64x2.62
201	Column tube
202	Tie rod
203	Flange, bottom
204	Flange, top
211	O-ring 673.1x9.525
213	Hex Head Screw M20x60
221	Hinge lower asm
231	Hex head screw ISO 4017 M16x45 A4-70 (SSI column tube)
232	Hex head screw ISO 4017 M16x50 A4-70 (SS column tube)
301	Bed support bottom 600, 10 or 20 µm
302	Bed support adapter 600, 10 or 20 µm
401	Distributor bottom 600
402	Distributor adaptor 600
403	Bed support screw bottom 300-600
404	Bed support screw adaptor 300-600
405	Distributor adaptor ring 600
406	Snap ring 600
409	Fastener, bottom
411	T-piece, 3TC, ASME BPE DT- 18, 1/2in, SF5
412	Pressure Transmitter
413	O-ring 26.7x1.78
414	O-ring 600x10
415	O-ring 556.86x6.985
416	O-ring 506.86x6.985

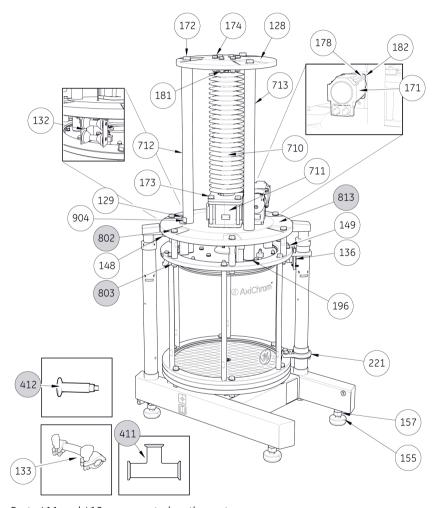
Position	Name
417	O-ring 30x3.5
421	Hex Socket Head Cap Screw ISO 4762 M8x25 A4-70
422	Washer ISO 7089 8.4x16x1.6 A4
423	Parallel Pin ISO 2338 6m6x16 A2
431	Hex Nut ISO 4032 M10 A4-70
432	Washer ISO 7089 10,5x20x2 A4
501	Tube Mobile Phase, bottom
502	Tube Slurry inlet/outlet, bottom
503	Tube Rinse, bottom
504	Connector top inlet
505	End cap
506	TC clamp for TC 25
507	TC-gasket, TC25 ID15, EPDM
508	Inner Connector Rinse (only used with PP tubes on 300-600 columns)
509	O-ring (only used with PP tubes on 300-600 columns)
511	Top Inlet/outer body
512	O-ring 16x3 EPDM70
513	Parallel Pin ISO 2338 4M6×16 A2
516	Valve body, outer
517	Valve body, inner
518	O-ring holder
519	Piston
521	Pneumatic cylinder
537	Washer
541	O-ring 125x5
542	Top inlet
543	Valve

Position	Name
601	O-ring 14x3
602	O-ring 48x3.5
603	O-ring 21.2x3
604	O-ring 27x4
607	O-ring 48x3.5
611	O-ring 48x3.5
612	O-ring 27x4
614	O-ring 48x3.5
616	O-ring 14x1.78
617	O-ring 14x1.78
618	O-ring 8x3
624	O-ring 14x3
628	O-ring 565x11
641	Scraper
710	Bellows
711	Screw and gear
712	Adapter rod with level scale
713	Adapter rod
801	Hex Head Bolt M20×110
802	Hex Head Bolt M24×300
803	Hex Nut M24
811	Bottom backing plate
812	Adapter backing plate
813	Lid
814	Adapter stop
904	Level display label

## A.2 Columns with any bed support material

#### Note:

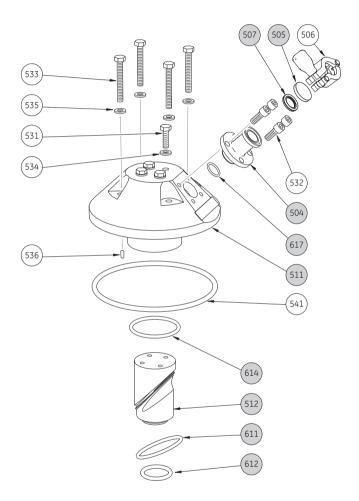
- The numbers correspond to position numbers in Material Conformity and Spare Parts
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



Parts 411 and 412 are mounted on the system.

Part 133 (Storage solution fill hose) is dismounted after commissioning.

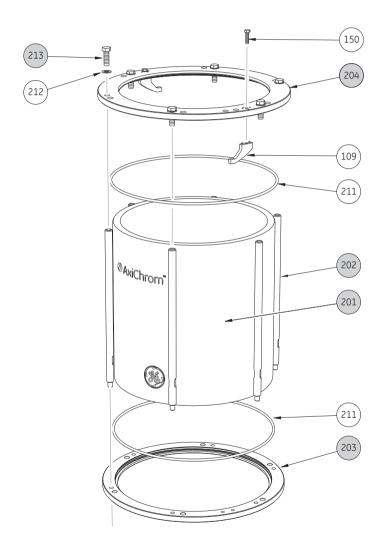
- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



Part 536 is not included in newer inlet/outlets.

## Columns with acrylic tubes

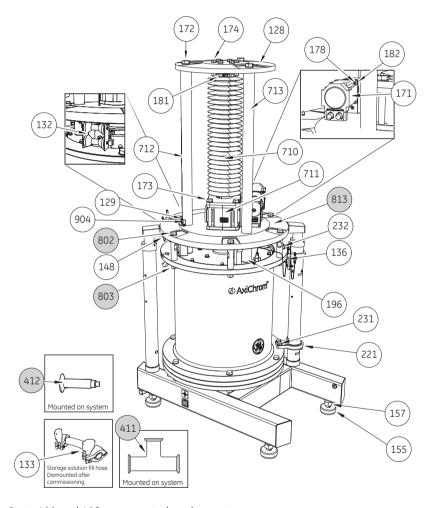
- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



# Columns with stainless steel tubes

Note:

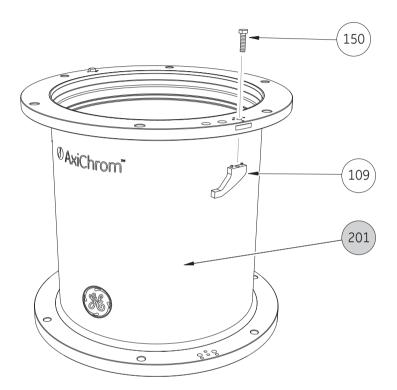
- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



Parts 411 and 412 are mounted on the system.

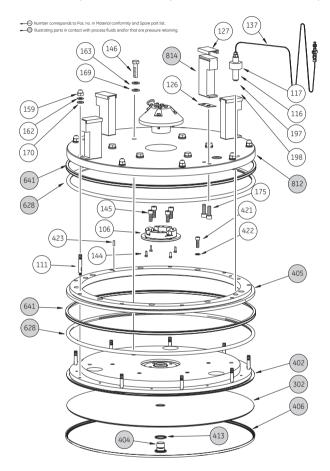
Part 133 (Storage solution fill hose) is dismounted after commissioning.

- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.

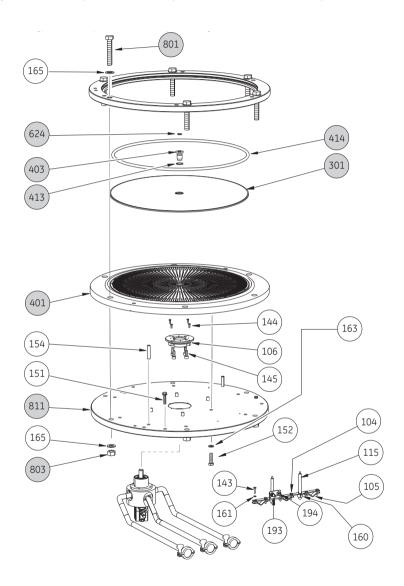


# A.3 Columns with plastic bed supports

- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.

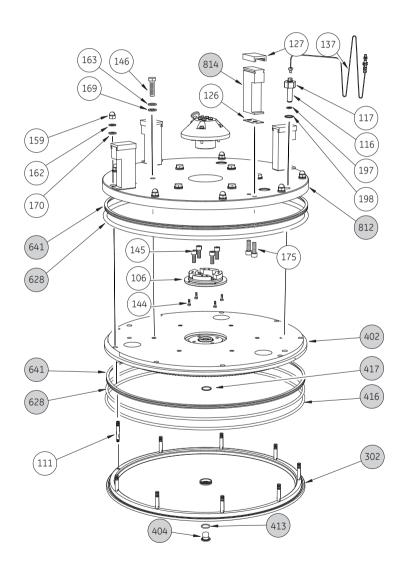


- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.

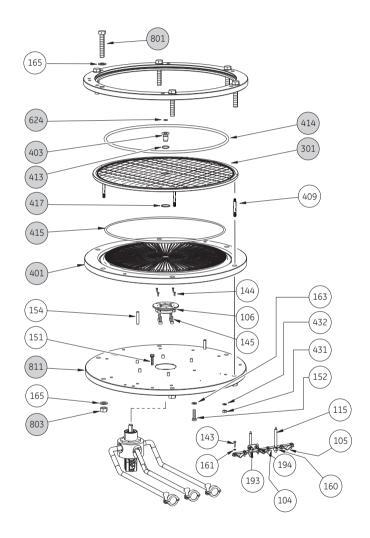


## A.4 Columns with stainless steel bed supports

- The numbers correspond to position numbers in Material Conformity and Spare Parts
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.

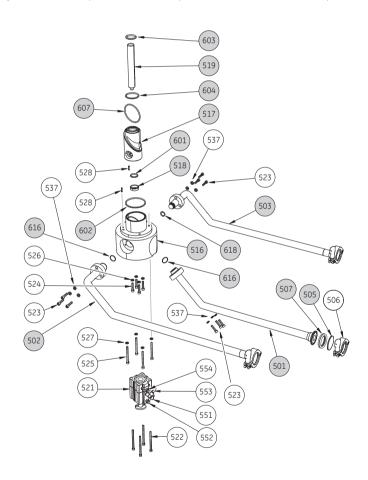


#### A.5 Media valve and tubes

#### Columns with stainless steel tubes and 800 and 1000 columns with PP tubes

Note:

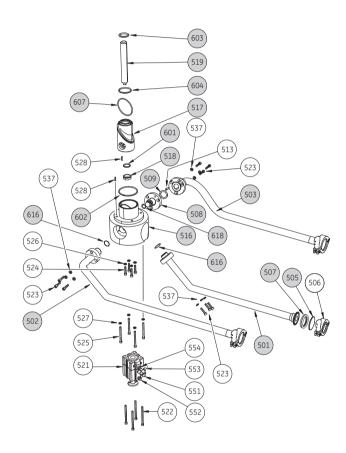
- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



#### 300-600 columns with PP tubes

Note:

- The numbers correspond to position numbers in Material Conformity and Spare Parts.
- Grey circles indicate parts which retain pressure or are in contact with process fluids.



## Appendix B Packing buffer information

## Packing buffers and packing factors

#### **Packing values**

Note:

The Packing Factor range used in the verified packing procedure in the Master or UNICORN is PF  $\pm 0.03$ . The range has been verified to give stable efficient packed beds. Media not listed here may be available in UNICORN or AxiChrom Master.

Туре	Media	Packing buffer	Packing factor	Packing velocity (cm/h)
Affinity	Blue Sepharose™ Fast Flow	Water/ 0-20% EtOH	1.15	60
	Heparin Sepharose 6 Fast Flow	Water/ 0-20% EtOH	1.15	60
	MabSelect	Water/ 0-20% EtOH	1.15	60
	MabSelect SuRe	Water/ 0-20% EtOH	1.15 <sup>1</sup>	60
	MabSelect SuRe LX	Water/ 0-20% EtOH	1.15 <sup>1</sup>	60
	MabSelect Xtra™	Water/ 0-20% EtOH	1.15	60

When calculating the amount of media to be packed use the Compression Factor 1.1 instead of the Packing Factor above which is used during packing.

#### B Packing buffer information

Туре	Media	Packing buffer	Packing factor	Packing velocity (cm/h)
Anion exchange	Capto adhere	Water/ 0-20% EtOH	1.10	60
	Capto DEAE	Water/ 0-20% EtOH	1.10	60
	Capto DEAE	Any salt solution	1.15	60
	Capto Q	Water/ 0-20% EtOH	1.10	60
	Capto Q	Any salt solu- tion	1.15	60
	DEAE Sepharose Fast Flow	Water/ 0-20% EtOH	1.15	60
	Q Sepharose Big Beads	Water/ 0-20% EtOH	1.18	60
	Q Sepharose Fast Flow	Water/ 0-20% EtOH	1.15	60
	Q Sepharose High Performance	Water/ 0-20% EtOH	1.15	60
	Q Sepharose XL	Water/ 0-20% EtOH	1.15	60

Туре	Media	Packing buffer	Packing factor	Packing velocity (cm/h)
Cation exchange	Capto MMC	Water/ 0-20% EtOH	1.13	60
	Capto MMC	Any salt solution	1.16	60
	Capto S	Water/ 0-20% EtOH	1.10	60
	Capto S	Any salt solu- tion	1.20	60
	CM Sepharose Fast Flow	Water/ 0-20% EtOH	1.15	60
	SP Sepharose Big Beads	Water/ 0-20% EtOH	1.18	60
	SP Sepharose Big Beads	Any salt solu- tion	1.20	60
	SP Sepharose Fast Flow	Water/ 0-20% EtOH	1.15	60
	SP Sepharose Fast Flow	Any salt solution	1.15	60
	SP Sepharose High performance	Water/ 0-20% EtOH	1.15	30
	SP Sepharose High performance	Any salt solu- tion	1.17	30
	SP Sepharose XL	Water	1.15	60
	SP Sepharose XL	Any salt solu- tion	1.15	60
Hydrophobic	Butyl -S Sepharose 6 Fast Flow	10-20% EtOH	1.15	60
interaction	Butyl Sepharose High Performance	10-20% EtOH	1.15	30
	Phenyl Sepharose High Performance	10-20% EtOH	1.15	30

#### B Packing buffer information

Туре	Media	Packing buffer	Packing factor	Packing velocity (cm/h)
Metal affinity	Chelating Sepharose Fast Flow	Water/ 0-20% EtOH	1.15	60
	IMAC Sepharose 6 Fast Flow	Water/ 0-20% EtOH	1.15	60
	Ni Sepharose 6 Fast flow	Water/ 0-20% EtOH	1.15	60
Size exclusion	Superdex™	Water/ 0-20% EtOH	1.15	30
Custom	Custom (default settings)	Custom	1.0	30

### **Unpacking values**

All values are in cm/h.

Media	Expand flow	Unpacking expand speed	Unpacking flow	Adapter unpacking speed
Capto, MabSelect, Fast Flow, XL, Big Beads	75	60	30	60
High Performance, Superdex	40 (cycle 1) 60 (cycle 2)	30	30	60 (cycle 1) 30 (cycle 2)
Custom (default settings)	75	60	30	60

# Appendix C Accessories and additional literature

#### **Tubings**

Article name	Code no.
Tube S-Cor 3/8" (ID=9.5), TC25 ID=7.75, 2000 mm	28-9377-58
Tube S-Cor 3/8" (ID=9.5), TC25 ID=7.75, 5000 mm	28-9381-24
Tube S-Cor 1/2" (ID=12.7), TC25 ID=9.4, 2000 mm	28-9381-25
Tube S-Cor 1/2" (ID=12.7), TC25 ID=9.4, 5000 mm	28-9381-26
Tube U-Cor 3/4" (ID=19.1), TC25 ID=15.75, 2000 mm	28-9381-27
Tube U-Cor 3/4" (ID=19.1), TC25 ID=15.75, 5000 mm	28-9381-29
Tube U-Cor 1" (ID=25.4), TC50 ID=22.1, 2000 mm	28-9381-36
Tube U-Cor 1" (ID=25.4), TC50 ID=22.1, 5000 mm	28-9381-37
Tube U-Cor 1½" (ID=38.1), TC50 ID=34.8, 2000 mm	28-9381-38
Tube U-Cor 1½" (ID=38.1), TC50 ID=34.8, 5000 mm	28-9381-39

#### **T-junctions**

Article name	Code no.
Tee 3/8" (3xTC25) ID=7.75	28-9381-67
Tee 1/2" (3xTC25) ID=9.4	28-9381-68
Tee 3/4" (3xTC25) ID=15.75	28-9381-69
Tee 1" (3xTC50) ID=22.1	28-9381-70
Tee 1½" (3xTC50) ID=34.8	28-9381-71

#### Reducers

Article name	Code no.
Reducer (TC25/ID=7.75 - TC25/ID=9.4)	28-9381-73
Reducer (TC25/ID=9.4 - TC25/ID=15.75)	28-9381-74
Reducer (TC25/ID=15.75 - TC50/ID=22.1)	28-9381-75
Reducer (TC50/ID=22.1 - TC50/ID=34.8)	28-9381-76

## TC gaskets

Article name	Code no.
TC-Gasket 25 / ID=10.5 (For tube ID=7.75)	28-9381-86
TC-Gasket 25 / ID=12 (For tube ID=9.4)	28-9381-87
TC-Gasket 25 / ID=15.8 (For tube ID=15.75)	28-9381-88
TC-Gasket 50 / 22.1 (For tube ID=22.1)	28-9668-86
TC-Gasket 50 / 34.9 (For tube ID=34.8)	28-9381-89

## Clamps

Article name	Code no.
Clamp TC25	28-9381-90
Clamp TC50	28-9381-91

#### Wheel kits

Article name	Code no.
AxiChrom 300 wheel kit	28-9381-92
AxiChrom 400 wheel kit	28-9381-93
AxiChrom 450 wheel kit	28-9554-29
AxiChrom 600 wheel kit	28-9381-94
AxiChrom 800 wheel kit	28-9381-96
AxiChrom 1000 wheel kit	28-9381-97

## **Grounding kit**

Article name	Code no.
Grounding kit	18-1157-87

#### **Toolkit**

Article name	Code no.
Tool kit Axichrom 300-1000	28-9382-03
Plastic mallet	28-9820-32
Removal tool	28-9820-31

#### Safety valves

Article name	Code no.
Safety Valve 4bar ID=9.4	28-9382-00
Safety Valve 4bar ID=15.75	28-9382-01
Safety Valve 4bar ID=22.1	28-9382-02

### Rupture disc and holder

Article name	Code no.
Rupture disc kit 4.2 bar for AxiChrom 300-600 (contains one special T-junction and one rupture disc)	29-0139-97
Rupture disc 4.2 bar, TC50	29-0139-98

For rupture discs used on AxiChrom 800-1000 the T-junction 28-9381-70 can be used.

#### Gemü valves

Article name	Code no.
Gemü-valve, ID=7.75, 2xTC25	28-9382-05
Gemü-valve, ID=9.4, 2xTC25	28-9382-06
Gemü-valve, ID=15.75, 2xTC25	28-9382-07
Gemü-valve, ID=22.1, 2xTC50	28-9382-08
Gemü-valve, ID=34.8, 2xTC50	28-9382-09

#### Valve holder kit

Article name	Code no.
Valve holder kit	29-0137-10

#### Media stirrer

Article name	Code no.
Media stirrer 80 mm	28-9191-03
Media stirrer 150 mm	28-9191-04

#### **Additional literature**

Article name	Code no.
Sanitization and endotoxin clearance in AxiChrom columns Application note	28-9290-42
Column efficiency testing Application note	28-9372-07

# Appendix D Crate weights and dimensions

Typical weights and dimensions for the different crate sizes are listed in the table below.

Column diameter [mm]	Column bed heights [mm]	Crate height [mm]	Crate length [mm]	Crate width [mm]	Empty crate weight [kg]	Total weight (crate+col- umn) <sup>1</sup> [kg]
300	100 to 300	1920	970	1495	160	574/580
300	100 to 500	2180	970	1495	165	599/605
400	100 to 300	1920	970	1495	160	611/620
400	100 to 500	2180	970	1495	165	636/645
450	100 to 300	1920	1090	1480	180	879/890
450	100 to 500	2180	1090	1480	185	934/945
600	100 to 300	1875	1100	1395	195	1013/1030
600	100 to 500	2135	1100	1395	205	1088/1105
800	100 to 300	2190	1440	1800	350	2472/2500
800	100 to 500	2450	1440	1800	360	2572/2600
1000	100 to 300	2240	1650	2040	440	2957/3000
1000	100 to 500	2500	1650	2040	450	3087/3130
Master		1300	770	700	65	140

<sup>1</sup> Weights are given for columns with plastic bed support/stainless steel bed support

**Note:** Some crate weights are estimates.

# Appendix E Column and AxiChrom Master weights and dimensions

The actual weight and dimensions for the individual column is given in the documentation. Typical weights and dimensions for the different column sizes are listed in the table below. The min. and max. heights refer to the height of the column with the adapter in its lowest position for transportation, and with the adapter in its highest position, for example for maintenance work.

# Column weights and dimensions (acrylic column tube)

Column diameter [mm]	Column bed heights [mm]	Footprint (length × width) [mm]	Min. height [mm]	Max. height [mm]	Weight of empty column <sup>1</sup> [kg]
300	100 to 300	1110 × 520	1450	2220	414/420
300	100 to 500	1110 × 520	1710	2720	434/440
400	100 to 300	1110 × 600	1425	2220	451/460
400	100 to 500	1110 × 600	1685	2720	471/480
450	100 to 300	1110 × 620	1470	2330	699/710
450	100 to 500	1110 × 620	1730	2750	749/760
600	100 to 300	1180 × 780	1570	2340	818/835
600	100 to 500	1180 × 780	1830	2860	883/900
800	100 to 300	1470 × 1080	1720	2630	2122/2150
800	100 to 500	1470 × 1080	1980	3150	2212/2240
1000	100 to 300	1720 × 1300	1875	2650	2517/2560
1000	100 to 500	1720 × 1300	2135	3170	2637/2680

<sup>1</sup> Weights are given for columns with plastic bed support/stainless steel bed support

# Column weights and dimensions (stainless steel column tube)

The table below lists column tube weights and the weights of empty columns and crate+column for columns with stainless steel tubes. All weights are given in kg.

Column	Column tube	Empty column <sup>1</sup>	Crate+column <sup>2</sup>
300 short tube	73	431/437	591/597
300 long tube	89	454/460	619/625
400 short tube	92	481/490	641/650
400 long tube	113	508/518	674/683
450 short tube	106	730/741	910/921
450 long tube	130	786/797	971/982
600 short tube	178	864/881	1059/1076
600 long tube	209	930/947	1135/1152
800 short tube	351	2231/2259	2581/2609
800 long tube	394	2318/2346	2678/2706
1000 short tube	584	2738/2781	3178/3221
1000 long tube	637	2841/2884	3291/3334

<sup>1</sup> Weights are for columns with plastic bed support/stainless steel bed support.

## AxiChrom Master weights and dimensions

Parameter	Value
Weight [kg]	73
Length [mm]	670
Width [mm]	590
Height [mm]	1090

Weights are for columns with plastic bed support/stainless steel bed support.
Some crate weights are estimates.

## Appendix F Decontamination report

#### **Decontamination Report**

- 1. Equipment that is returned, for service or any other reason when personnel connected to GE Healthcare must handle the equipment (at any location), should be cleaned.
- 2. This form must be used to log the decontamination of the equipment.
- 3. A completed copy of this form should be faxed or sent by first class post to the person who will come in contact with the equipment, to ensure that he/she has the information before handling the equipment.
- 4. Failure to complete the form or comply with the procedure will lead to costs for decontamination and delays in handling the equipment.

	Address:Fax number:
6. Please complete the f	'ollowing sections
6.1 Equipment type	6.2 Serial number
6.3 Details of substances	used on the inside/outside of the equipment
6.3.1 Substance nam	
	be taken in handling these substances:
	en in the event of human contact:
6.3.4 Cleaning fluid to	be used if residue of chemicals is found during handling:
	vant information:
7. If any substances use	ed in the system are hazardous or toxic, these must be highlighted under 6.3.1.
with toxic and hazard	the equipment has been thoroughly cleaned, whether or not the equipment has been in contact lous substances, and that the equipment has been filled with a suitable solution. I have also atof the cleaning method.
Storage solution in ed	quipment:
Signed:	
Position:	Date:

Document number: 04-0051-11

Edition: AA



For local office contact information, visit www.gelifesciences.com/contact

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